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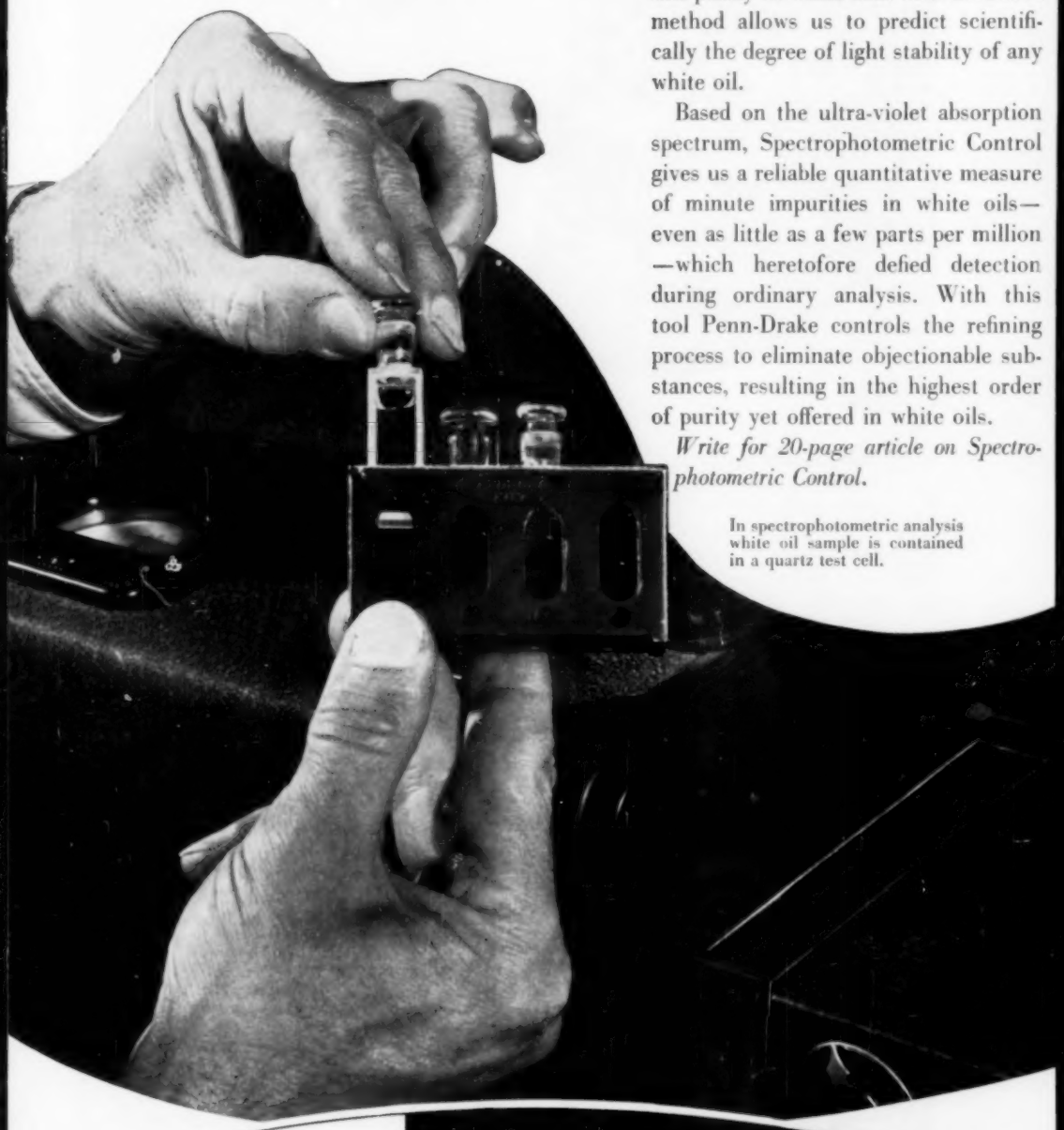

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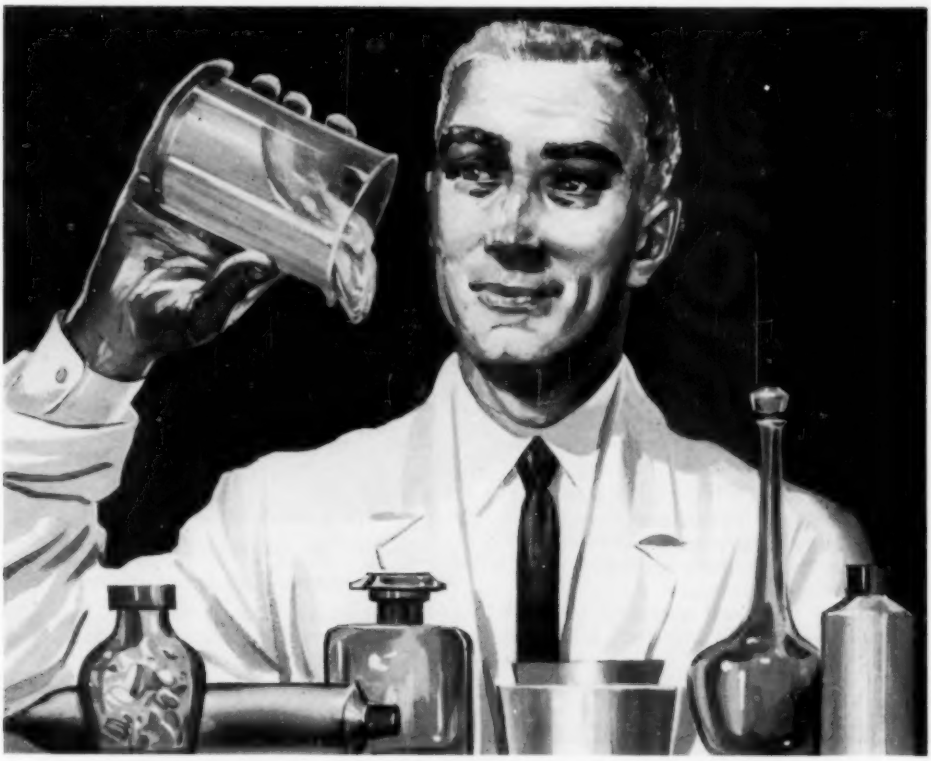

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The next annual meeting of the American Pharmaceutical Association will be held in Los Angeles, California, April 20 to 26, 1958. Titles and abstracts (not to exceed 200 words) of papers must be submitted to the appropriate Section secretary not later than February 1, 1958, or preferably earlier, in order to assure a place on any Section program.

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1958 Iodine Research Award Nominations Requested Prior to January 1, 1958

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1958 Chilean Iodine Educational Bureau, Inc., Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1958 Award, *nominations must be received on or before January 1, 1958.*

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION's Council. The present committee includes Justin L. Powers, chairman; F. F. Blicke, E. A. Brecht, John E. Christian, Stanley G. Mittelstaedt, Lloyd M. Parks, and Linwood F. Tice.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its tenth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Missouri, was the recipient of the fifth award. Dr. Jack Gross, Associate Professor of Anatomy, State University of New York, College of Medicine in Brooklyn, received the sixth award and Dr. Dominick Papa, Schering Corporation, Bloomfield, N. J., received the seventh award. The eighth award went to Dr. John E. Christian, Professor of Pharmaceutical Chemistry, School of Pharmacy, Purdue University, Lafayette, Indiana. The ninth award was conferred on Dr. James O. Hoppe, Research Pharmacologist, Sterling-Winthrop Research Institute, Rensselear, New York, during the 1957 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

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VOLUME XLVI — *OCTOBER 1957* — NUMBER 10
CONSECUTIVE NO. 20

A Comparative Study of the Metabolism in the Human Body of Some Isomers of Tartaric Acid*

By CHARLES W. BAUER and ROBERT WHITON PEARSON

A method of analysis of urine for tartaric acid has been developed. A color comparison curve for the determination of tartaric acid in urine has been made for tartaric acid-metavanadate mixture. The metabolism of the different isomers of tartaric acid in a human being has been determined. The percentage of tartaric acid eliminated in the urine after intramuscular injection is only slightly greater than that after oral ingestion of tartaric acid. The D (−) isomer of tartaric acid is more readily metabolized than the L (+) isomer. The DL isomer taken by mouth gives slightly higher values in the urine than does the D (−) isomer, but it is considerably lower than the L (+) isomer.

THE METABOLISM OF TARTARIC ACID in the animal body has been investigated by many workers. The animals used include rabbits, guinea pigs, cats, rats, and human beings.

The administration was done orally, subcutaneously, and intramuscularly.

The isomers used in some of the animals were the L-(+)-, or ordinary tartaric acid, D-(−)-, and DL forms.

The methods of analysis were those of precipitation or of colorimetric determination. In the precipitation methods the tartaric acid was recovered either as calcium tartrate or as potassium hydrogen tartrate. In the colorimetric method sodium metavanadate was used, and the color produced was read by means of standards in comparison tubes.

All the earlier workers come to the conclusion that tartaric acid is almost completely oxidized. The workers who reached this conclusion were Pohl (1), Brion (2), and Neuberg and Saneyoshi (3). These investigations were made between 1896 and 1911.

The view that tartaric acid is in great part metabolized in the body is frequently quoted from Dakin's book.[1]

In 1925 Simpson (4) stated that up to 88 per cent of subcutaneously administered tartaric acid in dogs, cats, and rabbits is recovered in the urine. He said: "It is therefore unlikely that tartaric acid is oxidized in the tissues."

In 1931 Underhill, Peterman, Jaleski, and Leonard (5) came to the conclusion that tartaric acid is largely unchanged in the animal body. The same conclusion was reached by Finkle (6) in 1933 on finding that only 20 per cent of the tartaric acid taken by mouth is eliminated in the urine but that 90 per cent administered by injection is eliminated. He assumed that about

* Received May 3, 1957, from the Massachusetts College of Pharmacy, Boston 15.
Presented to the Scientific Section, A. PH. A., New York meeting, April–May, 1957.
This paper is based on a thesis presented to the Graduate Council of the Massachusetts College of Pharmacy by Robert Whiton Pearson, in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

[1] H. D. Dakin, *Oxidations and Reductions in the Animal Body*, London, 1922, 2nd ed.

80 per cent of the orally administered tartaric acid is destroyed by intestinal bacteria and reasoned that the amounts of tartaric acid injected into animals used by the earlier workers produced severe injury to the renal tubules that delayed or prevented excretion of much of the tartaric acid. This latter view was first expressed by Underhill, Wells, and Goldschmidt (7). Finkle reasoned that the methods for the determinations used by the earlier workers were not so accurate as the method he used.

Finkle's work has generally been accepted as reliable. But no one has either confirmed or denied the present view based on Finkle's work, that tartaric acid is almost completely eliminated unchanged.

The present view expressed in *The United States Dispensatory*, 25th edition, page 1388, is that the tartaric acid which is absorbed in the intestine is eliminated unchanged in the urine. It also states that acid tartrates in large doses are markedly injurious to the kidneys.[2]

In this investigation the question of the metabolism of tartaric acid in a human being was studied. To reduce the variables to a minimum the following conditions were closely observed: (*a*) A reliable test method was followed; (*b*) The results following the different routes of administration were compared; (*c*) Three isomers of tartaric acid were studied and their metabolism compared; (*d*) All tests were made on the same subject.

EXPERIMENTAL DATA

At the beginning of this investigation a search was made for a reliable assay method for tartaric acid. The precipitation methods used by the earlier workers were found unreliable. Underhill, Peterman, and Krause (8) had previously shown that the precipitation methods used by Pohl (1), Brion (2), Neuberg and Saneyoshi (3), in which the tartaric acid is recovered as calcium tartrate, give low values. Simpson (4) improved the precipitation method by recovering the tartaric acid as potassium hydrogen tartrate. The potassium hydrogen tartrate method of precipitation is the accepted method adopted by the A.O.A.C.[3] for the determination of tartaric acid in wines. The A.O.A.C. method was tried and was found to be unsatisfactory for the determination of tartaric acid in urine. The amount of tartaric acid eliminated in the urine is less than the solubility of the precipitate.

The colorimetric method, used by Underhill, *et al.* (8), and developed independently by Finkle, was also tried. In this method tartaric acid reacts with sodium metavanadate to give a series of color changes from yellow to red. It was found that urine contains substances that interfere with the formation of the color obtained in the reaction of tartaric acid with sodium metavanadate. One of these substances was found to be ascorbic acid. A number of changes were made in Underhill's method so that it gave more nearly reproducible results.

Modified Sodium Metavanadate Method.—Underhill used color comparison tubes to make his determinations. In the present investigation it was found that a Cenco Photelometer with a yellow filter could be used to minimize the human error in the matching of colors. It was also found that the concentration of the sodium metavanadate could be reduced from 5 to 2% in order to lower the color of the reagent blank but still to have a sufficient amount of reagent to react with the tartaric acid. Furthermore, transmittancy readings were made at different intervals of time in order to determine the ideal duration of time in which to take the readings. The readings remained constant for twenty minutes after a reaction period of fifty-five minutes. Hence all the transmittancy readings were made after a period of one hour.

Standard solutions containing concentrations of tartaric acid, calculated to be present in the urine samples if the tartaric acid injected is eliminated unchanged, were added to the sodium metavanadate reagent. The reaction times with the corresponding transmittancy readings were determined. The results are shown in Table I.

TABLE I.—THE EFFECT OF TIME OF TRANSMITTANCY READINGS OF STANDARD SOLUTIONS OF TARTARIC ACID AND SODIUM METAVANADATE

Reading Time, Min. After Mixing	Transmittancy Readings	
	Solution I 22 mg. 50 ml.	Solution II 15 mg. 50 ml.
12	42	46
15	35	40
20	31	36
25	27	32
30	25	30
35	..	..
40	24	28
45	22	25
50	21	27
55	21	28
60	..	..
65	20	28
70	20	28
75	20	28
85	20	28
120	20	30

Data for the Establishment of a Comparison Curve.—One ml. of glacial acetic acid was added to four ml. of 2% sodium metavanadate. Varying amounts of a standard solution of L-(+)-tartaric acid were added to this mixture which was then diluted with distilled water to make 50 ml. The mixture was well shaken, and the transmittancy readings were taken after one hour. The results are shown in Table II.

The readings shown in Table II show that the reaction follows the Beer-Lambert law for concentrations between 10 and 20 mg. of tartaric acid per 50

[2] If the inference is correct that tartrates are only slightly absorbed into the blood stream when taken orally, then it is difficult to understand how they can be injurious to the kidneys. Furthermore, the most common acid tartrate is potassium hydrogen tartrate, which has not been tested. The statement about acid tartrates is not supported by experimental evidence.

[3] *Official Methods of Analysis, of the Association of Official Agricultural Chemists*, ed. 7, Association of Official Agricultural Chemists, Washington 4, D. C., 1950, p. 174.

ml. of final solution. The slope between these concentrations is shown in Fig. 1.

The effect of certain urinary substances on the tartaric acid-metavanadate mixture was studied. Urea had no effect. Glucose, which is present only in trace amounts in normal urine, had no effect. Ascorbic acid did affect the readings. Concentrations of ascorbic acid in amounts greater than is normally found in urine had a pronounced effect on the transmittancy readings. The effect of trace amounts of ascorbic acid was not determined. The results obtained are shown in Table III.

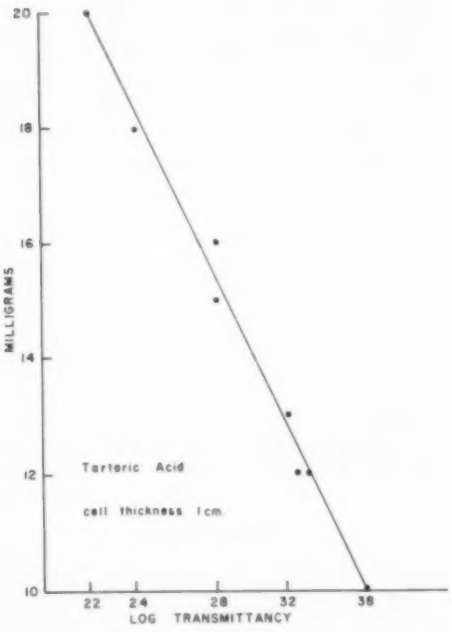

Figure 1.

TABLE II.—TRANSMITTANCY READINGS AFTER ONE HOUR WITH VARYING AMOUNTS OF TARTARIC ACID

Tartaric Acid, mg.	Transmittancy Readings
0	77
2.5	68
3	65
5	54
7.5	45
10	38
10	38
10	38
12	34
12	33
12	33
13	32
13	32
15	28
16	28
18	24
18	24
18	24
20	22
20	22
20	22
20	22
22	21
23	19
25	18

TABLE III.—THE EFFECT OF ASCORBIC ACID ON TRANSMITTANCY READINGS OF TARTARIC ACID-METAVANADATE MIXTURE

Absorbic Acid, mg.	Tartaric Acid, mg	Transmittancy Readings	
		Calcd.	Found
15	10	38	78
15	10	38	56
24	12	34	70
24	12	34	70
24	12	34	70

The oxidation of ascorbic acid to dehydroascorbic acid by being boiled with hydrogen peroxide, then being cooled to room temperature, did not eliminate the interference shown before oxidation. There were other interfering substances which were not identified.

The urine contains some substance or substances that prevent the use of the blue color produced in the reaction mixture by heat or exposure to bright sunlight. The green to purple colors produced by the interfering substances absorb heavily near the point of maximum absorbancy of the blue color.

PROCEDURE

After the ingestion of tartaric acid, or the intramuscular injection of tartaric acid as sodium tartrate, the urine was collected for at least twelve hours; the samples were pooled, and the total volume recorded. One hundred milliliters of urine were then decolorized by being boiled for four minutes with four grams of Norit;[4] they were filtered while hot, the filter paper was washed with hot distilled water and the filtrate was made faintly alkaline with normal sodium hydroxide with phenolphthalein as the indicator, and then cooled and diluted with distilled water to make 200 ml. This method gave a precipitate of insoluble phosphates. The precipitate was separated by filtration. The filtrate was assayed for tartaric acid.

Forty-five ml. of the cooled clear filtrate were mixed with four ml. of 2% sodium metavanadate and one ml. of glacial acetic acid. Transmittancy readings were made after one hour.

This process was followed after the ingestion of 2 Gm. of each of the following isomers of tartaric acid: L-(+)-, D-(−)-, and DL. The same process was followed after the intramuscular injection of about 750 mg. of the L-(+)- and D-(−)-isomers of tartaric acid in the form of sodium tartrate. After the ingestion or the injection of each form the subject was rested for one week before the next trial.

Each isomer was identified by its optical rotation and melting point.

The sodium tartrate was dissolved in 5 ml. of Water for Injection U. S. P. XV and was injected into the gluteal region. This injection of sodium

[4] Norit A obtained from Pfanstiehl Chemical Company, Waukegan, Ill.

tartrate gives the subject intense pain that lasts for about ten minutes, then gradually subsides.

The amount of tartaric acid administered, the route of administration, the isomer used, the volume of urine collected in a period of twelve hours or more, and the amount of tartaric acid recovered after each ingestion or injection are shown in Table IV.

TABLE IV.—TARTARIC ACID RECOVERED IN THE URINE AFTER ADMINISTRATION BY DIFFERENT ROUTES

Tartrate Expressed as Gm. of Tartaric Acid	Isomer	Route of Admin.	Volume of Urine	Tartaric Acid Recovered, %
2	L-(+)-	*Per os*	555	12.2
2	L-(+)-	*Per os*	370	6.0
2	L-(+)-	*Per os*	490	7.4
2	L-(+)-	*Per os*	198	0.4
0.765	L-(+)-	Intramusc.	475	12.4
0.750	L-(+)-	Intramusc.	475	6.35
0.750	L-(+)-	Intramusc.	730	16.5
0.720	L-(+)-	Intramusc.	540	11.7
2	D-(−)-	*Per os*	485	0.?
0.740	D-(−)-	Intramusc.	860	0.?
0.765	D-(−)-	Intramusc.	1100	1.86
2	DL	*Per os*	415	0.9
2	DL	*Per os*	605	2.0
2	DL	*Per os*	710	4.3
2	DL	*Per os*	305	0.5

DISCUSSION

The data collected in this investigation on the metabolism of tartaric acid in a human being do not agree with the findings reported by Underhill and with those reported later by Finkle.

Both Underhill and Finkle came to the conclusion that tartaric acid, even in small doses, is eliminated in great part in the urine, and that it is unchanged. The evidence presented in this investigation is that it is eliminated only in small part in the urine, though unchanged.

The method of analysis here used for the determination of tartaric acid in urine appears to be more reliable than those used by Underhill and by Finkle. The quantities of tartaric acid injected were kept small in order to avoid kidney damage, since Underhill and Finkle proved that small quantities did not produce kidney damage in human beings. It therefore seems unlikely that the small recovery of tartaric acid in these studies was due to delayed elimination because of kidney damage.

It is doubtful that the ascorbic acid in the urine of the experimental subject was responsible for the low results. This substance was not taken into consideration in Underhill's or in Finkle's determinations.

CONCLUSIONS

The different forms of tartaric acid administered by mouth or injected intramuscularly as sodium tartrate are largely metabolized when given in small amounts.

A 2% sodium metavanadate solution is superior to a 5% solution since it reduces the color of the reagent blank.

Waiting one hour after mixing the reagents before reading the colors reduces the variability in the intensity of the colors and gives more nearly reproducible results.

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Preparation of Metal Salts of Alkanesulfonic Acids*

By WILLIAM D. ROLL and GUSTAV E. CWALINA

A series of lanthanum salts of the lower alkanesulfonic acids were prepared for the purpose of evaluating their antiperspirant, astringent, and/or deodorant activity

CURRENTLY, ONE OF THE MORE EFFECTIVE antiperspirant compounds is thought to be aluminum chloride (1); however, this compound has been found to be quite irritant and deleterious to fabrics with which it is brought in contact. For that reason aluminum sulfate has found greater use even though it has relatively less antiperspirant activity. The aluminum ion is an effective protein coagulant and its antiperspirant activity is due to its ability to block the sweat glands because of this effect. This blocking action serves to check the flow of perspiration.

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Since the aluminum ion's antiperspirant activity is regarded as being due to its ability to block the sweat glands because it is an effective protein coagulant, it seems that the Hofmeister series provides theoretical grounds for the belief that lanthanum may be of superior value. The Hofmeister series for trivalent ions is listed by Kunin and Meyers (2) as follows: aluminum, scandium, yttrium, europium, samarium, neodymium, praseodymium, cerium, and lanthanum. This series is based on the ability of a salt to coagulate lyophilic sols such as albumin (3). The most effective coagulant in the series is lanthanum; therefore it would seem likely that this electropositive trivalent metal would be the most effective antiperspirant agent.

A series of antiperspirant salts was prepared by Collins (4) and evaluated on a molar basis with respect to the cation. The following compounds were studied and are listed in order of decreasing effectiveness: lanthanum sulfamate, lanthanum methionate, cerium methionate, cerium sulfamate, lanthanum chloride, aluminum chloride, zinc methionate, aluminum sulfamate, zinc sulfate, aluminum methionate, aluminum methanetrisulfonate, and aluminum sulfate.

EXPERIMENTAL

Sodium Alkanesulfonates.—The procedure employed in preparing all of the sodium salts was identical; therefore it will be necessary to describe it only once. Only the quantity of reagents used varied. Reference may be made to Table I for the yields obtained.

$$R—Br + Na_2SO_3 \rightarrow R—SO_3Na + NaBr$$

An aqueous solution of sodium sulfite in distilled water was placed in a three-necked reaction flask

TABLE I.—YIELD OF SODIUM ALKANESULFONATES PREPARED FROM ALIPHATIC BROMIDES AND SODIUM SULFITE

Salt	Yield, %
Sodium 1,1-ethanedisulfonate	81.5
Sodium 1,2-ethanedisulfonate	43.0
Sodium 1,1,2-ethanetrisulfonate	95.4
Sodium 1-propanemonosulfonate	98.0
Sodium 2-propanemonosulfonate	84.7
Sodium 1,2-propanedisulfonate	60.0
Sodium 1,3-propanedisulfonate	81.4
Sodium 1,2,3-propanetrisulfonate	83.5

and heated to boiling, employing a heating mantle which was controlled by a variac. To this reaction flask was fitted a reflux condenser and a mercury-sealed stirrer and to this a dropping funnel containing the alkyl bromide was attached. With constant rapid stirring, the alkyl bromide was added to the boiling solution of sodium sulfite over a two-hour interval. The mixture was refluxed for one hour after the disappearance of the organic layer. The aqueous solution was then cooled to 5° in an ice bath and the white lustrous crystals of the sodium salt of the acid appeared. They were filtered off on a Buchner funnel and recrystallized from boiling water. They were dried in an oven at atmospheric pressure for four hours at 140° and the yields determined.

Lanthanum Alkanesulfonates.—The procedure employed in preparing all of the lanthanum salts was identical; therefore, it will be necessary to describe it only once. Reference may be made to Table II

TABLE II.—DETAILS OF THE SYNTHESES OF LANTHANUM ALKANESULFONATES FROM CORRESPONDING SODIUM ALKANESULFONATES AND $LaCl_3 \cdot 7H_2O$

	Sodium alkanesulfonate, Gm.	$LaCl_3 \cdot 7H_2O$, Gm.	Lanthanum alkanesulfonate, Gm.	Yield, %
1.	2.0	2.12	2.44	96.1
2.	2.0	2.12	2.44	96.1
3.	2.0	2.23	2.20	90.0
4.	10.0	8.54	11.60	95.9
5.	4.0	3.38	2.60	54.3
6.	2.0	2.00	2.20	92.0
7.	2.0	2.00	1.60	66.9
8.	10.0	10.62	7.50	62.7

TABLE III.—RESULTS OF QUANTITATIVE ANALYSIS FOR LANTHANUM IN THE LANTHANUM ALKANESULFONATE SALTS

Salts	La Calcd., %	La Found, %
Lanthanum 1,1-ethanedisulfonate	32.9	33.1
Lanthanum 1,2-ethanedisulfonate	32.9	32.9
Lanthanum 1,1,2-ethanetrisulfonate	34.2	34.25
Lanthanum 1-propanemonosulfonate	27.2	26.6
Lanthanum 2-propanemonosulfonate	27.2	26.2
Lanthanum 1,2-propanedisulfonate	31.4	31.4
Lanthanum 1,3-propanedisulfonate	31.4	31.3
Lanthanum 1,2,3-propanetrisulfonate	33.1	32.8

for the quantities of reagents used and yields obtained in other syntheses.

$$3R—SO_3Na + LaCl_3 \cdot 7H_2O \rightarrow (R—SO_3)_3La + 3NaCl + 7H_2O$$

A hot saturated aqueous solution of the sodium salt of the acid was made in a 250-ml. Erlenmeyer flask. In another 250-ml. Erlenmeyer flask a saturated aqueous solution of $LaCl_3 \cdot 7H_2O$ was prepared. With constant stirring, the latter was added to the hot solution of the sodium salt. The reaction mixture was then allowed to stand overnight. The lanthanum derivative was filtered off with the aid of suction employing a Buchner funnel. The white salt was washed with distilled water until free of chloride ion and was then placed in an oven to dry at 100° and atmospheric pressure. Equations for the syntheses of the lanthanum alkanesu fonates follow:

1. $3CH_2CH(SO_3Na)_2 + 2LaCl_3 \rightarrow (C_2H_4S_2O_6)_3La_2$

2. $3CH_2(SO_2Na)CH_2SO_3Na + 2LaCl_3 \rightarrow (C_2H_4S_2O_6)_3La_2$
3. $CH_2(SO_3Na)CH(SO_3Na)_2 + LaCl_3 \rightarrow C_2H_3S_3O_9La$
4. $3CH_3CH_2CH_2SO_3Na + LaCl_3 \rightarrow (C_3H_7SO_3)_3La$
5. $3CH_3CH(SO_3Na)CH_3 + LaCl_3 \rightarrow (C_3H_7SO_3)_3La$
6. $3CH_3CH(SO_3Na)CH_2SO_3Na + 2LaCl_3 \rightarrow (C_3H_6S_2O_6)_3La_2$
7. $3CH_2(SO_3Na)CH_2CH_2SO_3Na + 2LaCl_3 \rightarrow (C_3H_6S_2O_6)_3La_2$
8. $CH_2(SO_3Na)CH(SO_3Na)CH_2SO_3Na + LaCl_3 \rightarrow C_2H_3S_2O_7La$

SUMMARY

1. A satisfactory procedure for synthesizing the lanthanum derivatives has been devised.

2. Eight new lanthanum salts of alkanesulfonic acids have been prepared in sufficient quantity for testing for antiperspirant activity.

3. In the near future the compounds will be tested to ascertain whether or not they have antiperspirant activity.

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The Electron Sterilization of Certain Pharmaceutical Preparations*

By GEORGE C. COLOVOS† and B. W. CHURCHILL†

Sterilization of a number of pharmaceuticals in the final package was accomplished with, in most cases, no loss in potency or increase in toxicity. A dose of 2×10^6 REP of cathode rays was adequate for the complete destruction of any bacterial contamination. The use of irradiated products clinically has shown no unfavorable reactions. Irradiated dog plasma when reinfused into dogs showed no untoward reactions. Cathode ray irradiations were also used for the selection of high yielding mutants of *Penicillium chrysogenum, Streptomyces fradiae,* and *Streptomyces erythreus.*

THE STERILIZATION of heat sensitive drugs has always been a costly and perplexing problem which has prompted the pharmaceutical industry to be on the lookout for less detrimental methods of sterilization. Cathode ray sterilization appears to offer great promise in this direction.

Edwards, *et al.* (1), Proctor and Goldblith (2) have investigated the sensitivity to cathode rays of *B. subtilis* spores under a variety of conditions. They have shown that within certain dosage limits, the percentage of spores surviving cathode ray irradiation appeared to exponential. Exposure of the cells at 44°, 0° and −65° resulted in a decreased kill at the elevated temperatures while low temperatures produced increased destruction.

No profound effect in the lethal activity of the rays was apparent when irradiations were performed at pH 5.4 and 9.0, nor in 0.25% and 2% concentrations of sodium chloride dissolved in a buffered medium. Five per cent skim milk solids did provide some protection for the *B. subtilis* spores. Maintaining the dosage constant but varying the length of time and the intensity of irradiation did not result in any significant change in the percentage of spores surviving.

The experiments designed to show the relationship between population of spores and the dosage producing complete kill demonstrate that exacting methods are necessary before the precise dose which will sterilize any given cell population can be determined. These data are of practical significance in that the dosage needed to sterilize certain pharmaceutical preparations may be predicted. While the data seem to indicate that almost any cell population may be sterilized by cathode rays, it must be borne in mind that some pharmaceutical preparations may show a potency loss under certain conditions of irradiation.

EXPERIMENTAL

In the work being reported by the authors, a two million volt Van de Graff Electron Accelerator (3, 4)

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† The authors gratefully acknowledge the cooperation of the members in the Research, Medical, Production, and Control Divisions of The Upjohn Company for their aid in the assays and clinical testing, and of Mr. D. G. Cummings for the irradiations.

with a scanner was used. The total possible maximum-beam current from this generator is approximately 250 microamperes at two million volts. This gives a 500-watt source of radiated ionizing energy.

A series of parenteral pharmaceutical products have been sterilized by exposure to cathode rays. Stability and toxicity have been used to determine the extent of the influence of the irradiation. The pharmaceuticals studied fall into the following categories: antibiotics, hormones and steroids, multivitamin preparations, anticoagulants, proteins, alkaloids, and sulfonamides.

Two million REP of cathode rays has been found to be an adequate dose for the complete destruction of bacterial contamination. Under the conditions of irradiation, some preparations did exhibit small but significant potency loss. This type of potency loss, however, may occur even with conventional methods of sterilization. Experience to date indicates that with some exceptions, drugs under the proper conditions are stable to sterilizing doses of cathode rays. For the few exceptions a change in formulation or physical state may make it possible to sterilize them by irradiation. It must be remembered, however, that each product is an individual problem.

The most extensive long range stability studies have been done on four penicillin and two multivitamin preparations. The procedure has been to take an adequate quantity of material from regular finished production lots; irradiate half of the samples and retain the other half as controls. Samples were then stored at 4°, 25°, and 40°. Tables I and II give the stability data on these products after storage for four years a 25°. As shown in the tables, over a four-year period the irradiated material is as st ble as the nonirradiated.

Not only must the product retain its potency after irradiation but there should be no appreciable increase in toxicity (5). In this study the toxicity of the irradiated product was compared to the nonirradiated material. All materials used were again taken from regular finished production lots.

One method which was used to measure increased acute toxicity was to compare the LD_{50} of the irradiated to the nonirradiated. In acute toxicity studies, comparable groups of mice received graded doses of the product to be tested. This was administered either subcutaneously or intravenously, depending upon the type of product.

Table III lists the same pharmaceutical preparations mentioned above that were studied in laboratory animals for indications of increased toxicity of the irradiated products. The ratio of LD_{50} is given as per cent of irradiated over nonirradiated. Under the conditions of the test there was no significant increase in acute toxicity due to irradiation.

TABLE I.—STABILITY OF IRRADIATED PRODUCTS AFTER FOUR YEARS STORAGE AT 25°

Product	Nonirradiated, mg.	Irradiated, mg.
Multivitamins No. 1		
Thiamine	7.5	7.3
Riboflavin	9.4	9.2
Pyridoxine	2.7	2.5
Ca-Pan	5.3	5.3
Ni-Amide	248.0	250.0
Multivitamins No. 2		
Thiamine	5.5	4.4
Riboflavin	8.3	8.3
Pyridoxine	6.1	6.1
Ca-Pan	50.0	51.0
Ni-Amide	250.0	245.0
Folic Acid	1.0	1.1
B_{12}	4.5 mcg.	4.3 mcg.

TABLE II.—STABILITY OF IRRADIATED PRODUCTS AFTER FOUR YEARS STORAGE AT 25°

Product	Nonirradiated, u/cc.	Irradiated, u/cc.
Procaine penicillin for aqueous injection	306,000	310,000
Procaine penicillin aqueous suspension	37,700	43,400
Procaine penicillin in oil	318,000	318,000
Procaine penicillin G and cryst. penicillin G in oil	405,000	401,000

TABLE III.—ACUTE TOXICITY DATA

Product	Ratio of LD_{50} in Per Cent of Irradiated over Nonirradiated
Multivitamins No. 1	102
Multivitamins No. 2	92
Procaine Penicillin for Aqueous Injection	102
Procaine Penicillin Aqueous Suspension	82
Cryst. Procaine Penicillin G	109
Cryst. Penicillin G Potassium	102
ACTH	100

For the determination of chronic toxicity a human dose was given to rabbits intramuscularly or intravenously, daily. For each sample to be tested, rabbits of comparable size were placed in five groups: Group 1 received 7 daily injections and was autopsied on the 8th day, Group 2 received 14 daily injections and was autopsied on the 15th day, Group 3 received 21 daily injections and was autopsied on the 22nd day, Group 4 received 21 daily injections and was autopsied on the 29th day, and Group 5 were the controls and received no injections. Comparison of the effects of irradiated and nonirradiated samples were made by weight change, the appearance of the animal, gross pathology, and micropathological examination of the tissues.

No significant weight or tissue differences were found in animals receiving either control or irradiated material.

Doses in excess of the sterilizing dose of cathode rays (up to 20×10^6 REP) resulted in attack on the aromatic ring in procaine and sodium penicillin G, and the β-lactam function in potassium penicillin G. The irradiation dose, as observed by infrared assay at which breakdown was first observed, appeared to be between 8.3 and 15×10^6 REP for the procaine salt, between 2.0 and 3.5×10^6 REP for the sodium salt, and between 5.0 and 8.3×10^6 REP for the potassium salt. Therefore, it is safe to say that the detectable irradiation decomposition dosages for procaine and potassium penicillin G are

safely removed from the sterilizing level of 2×10^6 REP but lies very close to the sterilizing dose for sodium penicillin G.

Table IV gives the acute toxicity data for these superirradiated penicillins.

TABLE IV.—ACUTE TOXICITY DATA

Product	REP $\times 10^6$	Ratio of LD_{50} in % of Irradiated over Nonirradiated
Penicillin G Procaine	8.3	134
	15.0	101
	20.0	111
Penicillin G Potassium	8.3	99
	15.0	102
	20.0	102
Penicillin G Sodium	8.3	74
	15.0	81
	20.0	61

The acute toxicities were done as described previously using five mice at each dose. An increase in toxicity is observed when the figures in the last column get smaller. The possible increased toxicity shown for sodium penicillin G correlates very well with the changes observed by infrared assay.

A cortisone acetate (25 mg./cc.) (6) suspension that had been irradiated at varying dosage levels up to 10×10^6 REP was given intramuscularly and subcutaneously to rabbits and rats respectively. Eight rabbits and ten rats were used in each of the tests. The animals were sacrificed as described above and a pathological examination was made. No differences were observed between the controls and those receiving irradiated material.

The use of irradiated products clinically has shown no unfavorable reactions. Two multivitamin preparations were administered to a total of 80 patients. With respect to pain on injection, tolerance, and patient acceptability there was no detectable difference between the irradiated and nonirradiated preparations.

Several thousand doses of ACTH have been administered to humans without any untoward reactions. Approximately 600 units of ACTH were administered over a period of ten to twelve days to each of four patients without any reactions. This material was administered subcutaneously, intramuscularly, and intravenously.

Liquid plasma, when treated with cathode rays at room temperature, undergoes very marked changes (7). Liquid plasma is affected more than dried plasma. Table V summarizes the plasma electrophoretic data. All electrophoretic analyses were run in V ronal buffer of pH 8.6 and ionic strength 0.1.

The electrophoretic pattern of irradiated liquid bovine serum shows a decrease in the concentration of the β- and γ-globulins with a corresponding increase in the α_2-globulin. The nonirradiated human plasma shows no difference between whole liquid plasma and lyophilized plasma. Lyophilized plasma receiving 1×10^6 REP shows no difference but there is an indication of breakdown when compared with nonirradiated. When treated with 2×10^6 REP, a tendency toward breakdown is observed.

The above results were of such interest that a quantity of normal dog plasma was irradiated and a like quantity was retained as a control for reinfusion into dogs. This plasma was stored in the frozen state until ready for use.

Both irradiated (2.75×10^6 REP) and nonirradiated dog plasma were infused into a series of dogs in the amount of 25 ml./Kg. A single infusion of normal dog plasma was given to two dogs. Two infusions of irradiated dog plasma were given to two dogs at three-week intervals. Seven dogs received irradiated dog plasma at three-week intervals and, one week after the last infusion, were given normal dog plasma. The side reactions observed after both control and irradiated plasma infusions were similar, consisting of lethargy, micturition, defecation, and occasional vomiting. All dogs were on their feet a few minutes after the infusions and appeared normal thereafter. The side-effects noted were attributable to the pentothal used to anesthetize the donor dogs, the citrate contained in the plasma, and the relatively large volume of plasma infused. All dogs were autopsied one to two weeks after the last plasma infusion. Pathological examination showed no gross lesions that

TABLE V.—PERCENTAGE DISTRIBUTION OF BOVINE AND HUMAN PLASMA PROTEINS

Material	Treatment	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	Fibrinogen	γ-Globulin
Bovine Serum	None	38.0	8.4	11.4	16.0	...	26.2
Bovine Serum	3.8×10^6 REP	39.1	9.2	46.7	1.4	...	3.6
Human Plasma	Liquid	56.0	3.7	11.6	12.4	5.3	11.0
Human Plasma	Lyophilized	57.7	4.1	10.6	11.9	6.8	9.0
Human Plasma	1×10^6 REP	53.8	5.6	11.8	12.2	5.6	11.1
Human Plasma	2×10^6 REP	51.2	8.4	17.2	8.6	5.8	8.8

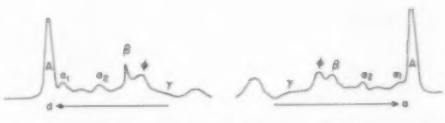

Fig. 1.—Electrophoretic pattern of normal dog plasma.

Fig. 2.—Electrophoretic pattern of cathode ray treated dog plasma.

TABLE VI.—PERCENTAGE COMPOSITION OF NORMAL AND CATHODE RAY TREATED DOG PLASMA

	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	Fibrinogen	γ-Globulin
Normal	42.9	7.5	14.1	11.5	16.7	7.3
Irradiated	24.0	17.2	32.6	6.8	14.2	5.3

TABLE VII.—A COMPARISON OF ANTIBIOTIC YIELDS WITH CATHODE RAY TREATED ISOLATES TO THE PARENT CULTURES

Organism Irradiated	REP	Organisms Killed, %	Antibiotic Produced	% Increase of Antibiotic Yield to Parent
P. chrysogenum	228,000	98.0	Penicillin G	36
S. fradiae	100,000	78.0	Neomycin	54
S. erythreus	200,000	97.6	Erythromycin	44

could be attributed to the administration of cathode ray treated dog plasma. Histopathologic examination showed no significant tissue differences in dogs receiving one infusion of normal dog plasma and those receiving two infusions of cathode ray treated dog plasma; however, splenic lesions (serum sickness type) were observed in the dogs that received two infusions of irradiated dog plasma and one infusion of normal dog plasma.

Table VI shows the electrophoretic data for normal and irradiated dog plasma. The change in the percentage composition of the components is quite marked.

Figures 1 and 2 show the electrophoretic patterns of the treated and untreated dog plasma.

Strains of *Penicillium chrysogenum*, *Streptomyces fradiae*, and *Streptomyces erythreus* have been treated with 2 Mev cathode rays. The purpose of this was to obtain variant isolates which would give higher antibiotic yields of penicillin G, neomycin, and erythromycin, respectively, than did the parent cultures. The strain of *P. chrysogenum* treated was Wisconsin Q 176, and the mutants selected had lost their ability to produce the yellow pigment chrysogenin. Sixteen pigmentless isolates were selected from the 2,000 which were screened. The highest yielding isolate is shown in Table VII. Many high yielding cultures were selected from irradiated populations of *S. fradiae* and *S. erythreus*. The isolates giving the best yields of neomycin and erythromycin are shown in Table VII.

SUMMARY

1. A series of pharmaceuticals were sterilized in the final container and it was found that with very few exceptions, a dose of 2×10^6 REP of cathode rays does not produce any loss in potency or increase in toxicity.

2. A dose of 2×10^6 REP of cathode rays was sufficient to destroy completely any bacterial contamination.

3. Irradiated products used clinically have shown no unfavorable reactions.

4. Dog plasma when treated with 2.75×10^6 REP of cathode rays and reinfused into dogs showed no untoward reactions. Pathological and histopathological examinations showed no significant differences in dogs receiving normal dog plasma and those receiving cathode-ray-treated dog plasma.

5. High yielding mutants of *Penicillium chrysogenum*, *Streptomyces fradiae*, and *Streptomyces erythreus* have been selected from cultures treated with cathode rays.

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Prediction of Stability in Pharmaceutical Preparations IV.*

The Interdependence of Solubility and Rate in Saturated Solutions of Acylsalicylates

By EDWARD R. GARRETT†

The stability of saturated solutions where the studied component undergoes solvolytic degradation is a function of both the solubility and the homogeneous rate. Prediction of stability in such preparations has been made from separate studies of the two factors and knowledge of the interaction relations. This may permit the choice of the more stable acylsalicylate from among acetyl-, diethylacetyl-, β-cyclopentylpropionyl-, and trimethylacetylsalicylic acid. For example, although the rate constants for the hydrolysis of β-cyclopentylpropionylsalicylic acid exceed diethylacetylsalicylic acid by factors of 20 to 35 at 26°, solubility considerations show that the overall rate of hydrolysis in saturated solutions is at least equal and even favors the former at pH values less than 3.7.

IT HAS BEEN SHOWN that saturated, fluid, pharmaceutical preparations will frequently show two different sequential kinetic orders (1, 2) in the mode of degradation of a studied component. If degradation in homogeneous solution is first order, the saturated solution is zero order.

Stability is a function of solubility and homogeneous rate, and can be predicted from separate knowledge of the factors. The proper choice of a drug modification on stability grounds on the assumption of equivalent biological activity can be made from the proper use of the relations among overall degradation, solubility, and solution rates. The general equation for the rate of a reaction of a component in saturated solution is:

$$\text{Amount/volume/time} = kc. = \text{constant} \quad \text{(Eq. 1)}$$

where k is the pseudo first-order rate constant in homogeneous solution, and c is the total solubility under the same conditions, such as pH.

This paper predicts the hydrolytic stability of saturated solutions of various acyl esters of salicylic acid, acetyl-, trimethylacetyl-, β-cyclopentylpropionyl-, and diethylacetyl- at all pH's from the derived kinetics (3) and from the calculation of solubility as a function of pKa.

EXPERIMENTAL

Several solutions were equilibrated with the various acyl esters of salicylic acid in excess at 25.6° for varying lengths of time dependent on their relative ease of hydrolysis. The minimum time of equilibration was one hour. In general, three separate equilibrations were run for different times for each solvent and ester. There was no significant correlation of apparent solubility with any greater time of equilibration (up to eighty hours in some cases). The excess ester was filtered off and the ultraviolet curves run on the Cary Model 14 at various dilutions and the pH of the equilibrated filtrate and diluted filtrate determined. The amount of salicylic acid in solution was determined from the absorptivity at 300 mμ and the absorbances at 276 mμ (maxima of acid forms of acyl esters of salicylic acid) and 260 mμ (an isobestic point of salicylic acid) were corrected for the contribution of this determined amount of salicylic acid. The 276 and 260 mμ corrected absorbances were used to calculate concentrations of the acyl esters of salicylic acid. The previously determined absorptivities of the esters at the appropriate pH of the diluted filtrates were used (see Table I).

Increases in salicylic acid content of filtrate had no significant effect on the apparent solubility of the acyl esters.

The solutions used in the equilibration were: (*a*) 0.1304 *M* HCl, 0.0625 *M* KCl of pH *ca.* 1.0, (*b*) 0.0209 *M* HCl, 0.0625 *M* KCl of pH *ca.* 1.7, (*c*) 0.7125 *M* acetic acid of pH *ca.* 2.4, (*d*) 0.07125 *M* acetic acid of pH *ca.* 3.0, (*e*) 0.1454 *M* acetic acid, 0.1046 *M* NaOH of pH *ca.* 4.4, (*f*) 0.1294 *M* KH_2PO_4 0.0948 *M* NaOH of pH > 5.0.

In the cyclopentylpropionylsalicylic acid case, additional alkali was added to maintain pH.

The average pH of the equilibrated filtrates and the average solubility at this pH (as averaged over the two wavelengths and three separate equilibrations) are given in Table II. Where absorptivities of esters had previously been determined in alcohol at different pH's, a change proportional to that of aspirin from the alcohol to water for a similar (pK − pH) value was made (Table I).

To check on the adherence of calculated pK values to those previously determined spectrophotometrically in water (3) (or corrected to zero alcohol, on the presumption that the same change in pKa′ with varying alcohol occurs with all acyl-

* Received May 15, 1957, from the Research Division, The Upjohn Company, Kalamazoo, Mich.
† The author is greatly indebted to Mrs. Kathryn S. Polzin for excellent technical assistance and to Mr. Maxton Murray for the supply of the esters.

TABLE I.—PERTINENT SPECTROPHOTOMETRIC DATA[a] OF ACYL ESTERS OF SALICYLIC ACID (a = ABSORPTIVITY IN GM./L.)

	Ethanol % 95%	Acid Form: a at 300 mμ	Acid Form: a at 268 mμ	Acid Form: a at 260 mμ	Acid Form: Max. Acid,	Acid Form: a at Max.	Salt Form: a at 300 mμ	Salt Form: a at 268 mμ	Salt Form: a at 260 mμ	Salt Form: a at Max.
Salicylic Acid	0.5	25.6	3.06	2.08	304	25.6	25.6	4.29	2.36	22.0
	20	25.6	2.67	1.92	304	26.2	25.6	4.24	2.25	22.1
	30	25.6	2.25	1.59	304	24.9	25.6	4.25	2.10	21.6
	40	25.6	2.55	1.82	304	26.9	25.6	4.11	2.12	22.9
Acyl Ester of Salicylic Acid:										
Acetyl-	0.5	0.44	5.07	3.97	277	6.26	0.29	3.24	3.55	2.39
	20	0.48	5.28	3.98	277	6.62	0.24	3.24	3.45	2.38
	30	0.30	4.97	3.67	277	6.08	0.18	3.21	3.48	2.39
	40	0.35	5.15	3.90	277	6.72	0.25	3.33	3.66	2.44
Diethylacetyl-	20	0.19	4.00	2.95	276	4.75	0.16	2.14	2.36	1.47
Trimethylacetyl-	0.5	0.50	4.35	3.41	277	5.21	0.36	2.40	2.63	1.55
β-Cyclopentyl-propionyl-	20	0.33	3.66	2.83	277	4.55	0.28	2.48	2.75	1.90

[a] Spectrophotometric data obtained on Cary Model 11.

TABLE II—SOLUBILITY DATA FOR VARIOUS ACYL ESTERS OF SALICYLIC ACID AT 25.6°

Acyl Ester of Salicylic Acid	pH	C Solubility (Moles/L)	pKa′ (actual)[a]	pKa′ (calcd.)[c]
Acetyl-	1.00	$1.89 \cdot 10^{-2}$	3.62	...
	4.33	$1.37 \cdot 10^{-1}$	...	3.5
Trimethylacetyl-	1.00	$9.73 \cdot 10^{-4}$	3.74	...
	5.95	$9.94 \cdot 10^{-2}$	...	3.9
Cyclopentylpropionyl-	1.00	$1.06 \cdot 10^{-4}$	3.91[b]	...
	6.66	$4.77 \cdot 10^{-2}$	...	4.0
Diethylacetyl-	1.00, 1.66	$2.80 \cdot 10^{-3}$	4.00[b]	... ...
	2.40	$4.29 \cdot 10^{-3}$	...	... ...
	2.97	$3.19 \cdot 10^{-3}$	...	3.8[d], ...
	4.39	$1.45 \cdot 10^{-2}$	...	3.8[d], 4.0[e]
	5.17	$1.06 \cdot 10^{-1}$	...	3.6[d], 3.8[e]

[a] As determined from spectrophotometric pKa′ determinations (3).
[b] The pKa′ values determined in alcohol are corrected to zero alcohol on the presumption that the same change in pKa′ occurs with all acylsalicylic acids as with aspirin.
[c] Based on the expression: $pKa' = pH + \log \frac{[HA]}{c - [HA]}$ where [HA] is presumed to be the concentration of acylsalicylic acid at high acidity, *viz.*, pH 1.0, and c is the experimentally determined concentration at the stated pH.
[d] Based on solubility determined in hydrochloric acid at pH's 1.00 and 1.66.
[e] Based on solubility determined in acetic acid at pH 2.40.

TABLE III.—SOLUBILITIES AND EXTENT OF HYDROLYSIS IN SATURATED SOLUTIONS OF VARIOUS ACYL ESTERS OF SALICYLIC ACID

Acyl Ester of Salicylic Acid → / pH	Acetyl-: C Solubility, Gm./L.	Acetyl-: Hydrolysis Saturated Solution, Gm./L./Day	Trimethylacetyl-: C Solubility, Gm./L.	Trimethylacetyl-: Hydrolysis Saturated Solution, Gm./L./Day	β-Cyclopentylpropionyl-: C Solubility, Gm./L.	β-Cyclopentylpropionyl-: Hydrolysis Saturated Solution, Gm./L./Day	Diethylacetyl-: C Solubility, Gm./L.	Diethylacetyl-: Hydrolysis Saturated Solution, Gm./L./Day
2.25	3.41	0.153	0.216	0.00113	0.0279	0.000651	1.014	0.00129
2.50	3.68	0.178	0.229	0.00175	0.0289	0.000780	1.047	0.00153
3.00	4.22	0.341	0.256	0.00397	0.0312	0.00108	1.115	0.00173
3.50	5.98	0.903	0.340	0.0111	0.0396	0.00224	1.34	0.00272
4.00	11.6	2.70	0.609	0.0338	0.0619	0.00786	2.03	0.00806
4.50	29.2	8.35	1.46	0.106	0.136	0.298	4.23	0.0292
5.00	85.2	26.3	4.16	0.334	0.371	0.108	11.2	0.104
5.50	263	83.4	12.65	1.05	1.11	0.359	33.1	0.351
6.00	822	263	39.5	3.32	3.46	1.16	102	1.13
6.50	2,590	831	124.6	10.5	10.81	3.66	322	3.61
7.00	8,220	2,640	394	33.3	34.2	11.7	1,010	11.5

salicylic acids as with aspirin) pKa′ data were calculated from solubility data by the expression:

$$pKa' = pH + \log \frac{[HA]}{c - [HA]} \qquad \text{(Eq. 2)}$$

where [HA] is the intrinsic solubility of the undissociated acyl ester of salicylic acid and may be presumed to be equal to the determined concentration of ester at saturation at pH = pKa′ − 2 or pH 1 to 2 and where c is the determined concentration at any pH.

These calculated pKa′ are also given in Table II

and are within ±0.1 of the actual pKa′ from the spectrophotometric determinations. In one case, however, the diethylacetylsalicylic acid solubilities at pH 1.00 and 2.40 were inconsistent, the latter 1.5 times the former. This is not readily comprehensible when it is realized that the pKa′ indicates only an increase in salt/acid ratio of 0.05 for this pH difference. The higher solubility value was used in all calculations so as to err on the safer side of predicting higher hydrolysis. A possible reason for this anomaly is that the acetic acid of the pH 2.4 (0.7 *M*) may increase apparent solubility by some mechanism of interaction.

The above Eq. 2, the actual pKa′, and the intrinsic solubility [HA] as determined at low pH values were used to calculate c in Gm./L., the saturation concentration of total ester at various pH's. These values are given in Table III as well as the Gm./L./day of hydrolysis of saturated solutions calculated from these values and the rate constants determined at 25°. Figure 1 plots these data for Gm./L./yr. hydrolysis of saturated solutions vs. pH. The determined and calculated solubilities (Table III) of acetylsalicylic acid at various pH values at 25.6° check the values obtained by Edwards using similar spectrophotometric procedures (4).

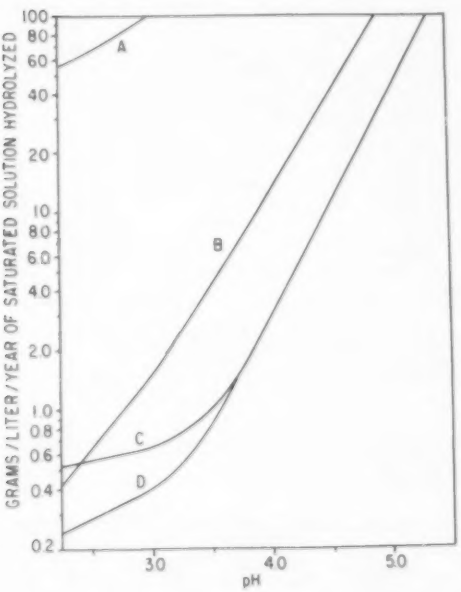

Fig. 1.—Grams per liter per year of acylsalicylate hydrolyzed in saturated solution at 26° as a function of pH. Acyl esters of salicyclic acid: A–acetyl; B–trimethylacetyl; C–diethylacetyl; D–β cyclopentylpropionyl.

DISCUSSION

Table III and Fig. 1 graphically demonstrate that comparison of rate constants for degradation in homogeneous media should not be the only criterion for choice of the properly stable modification. Diethylacetylsalicylic acid is much more stable to homogeneous solvolysis than β-cyclopentyl propionylsalicylic acid (3). For example, at 25° the former is 20 to 35 times more stable than the latter. Nevertheless, the solubility of the β-cyclopentylpropionylsalicylic acid is sufficiently low so that the net hydrolysis rate in saturated solution is equivalent to that of the relatively greatly soluble, but less reactive, diethylacetylsalicylic acid on the basis of Eq. 1.

Prediction of solubilities on the basis of this equation permits estimates of the stability of pharmaceutical preparations. The 100 Gm./L. preparations of diethylacetylsalicylic acid and β-cyclopentylpropionylsalicyclic acid will hydrolyze less than 1% in a year's time at 26° at pH values less than 3.5.

The fundamental premises of these predictions are: the solutions are aqueous with no other possible catalysts than those derived from water, only soluble species hydrolyze, and solubilization is nonrate determining.

SUMMARY

The hydrolysis rates of saturated solutions of acetyl-, trimethylacetyl-, β-cyclopentylpropionyl-, and diethylacetylsalicylic acids, have been calculated at all pH values from the relations among the separately experimentally determined values of pKa′, intrinsic solubility, and rate constants. It is shown that fast reacting materials in homogeneous solution may be more stable in saturated solutions than their less homogeneously reactive, more soluble analogs.

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The Solubility and Complexing Properties of Oxytetracycline and Tetracycline II.*

Interactions in Nonaqueous Solution

By EUGENE GANS† and TAKERU HIGUCHI

Anhydrous oxytetracycline base in chloroform and anhydrous tetracycline base in carbon tetrachloride form soluble complexes with N-methyl pyrrolidone, N-N′-dimethyl acetamide, gamma-valerolactone, and gamma-butyrolactone. At higher concentrations of gamma-butyrolactone, tetracycline forms an insoluble 1:1 complex, although there are indications that other polymeric species may be present. Comparison of the present N-methyl pyrrolidone interactions with the previous studies in aqueous solution suggests that the extent of interaction may vary inversely with the polarity of the media. With the exception of the tetracycline-gamma-butyrolactone interaction, and that of oxytetracycline-N-N′-dimethyl acetamide at higher concentrations, the interactions could be described by the equation: $y = ax^b$. When plotted logarithmically, the slopes of the straight lines indicate that the interactions were roughly first order with respect to the complexing agent.

EXPERIMENTAL EVIDENCE from previous studies in aqueous solution (1) indicated that the interactions might progress more favorably in the absence of water. The water molecules evidently play a definite role in the formation of inter- and intramolecular hydrogen bonding within the tetracyclines, since the hydrates of the individual bases release water of hydration only upon drying, *in vacuo*, at 80–100°. It would appear that the water molecules attached to the tetracyclines are oriented or bound in such a manner as to render complex formation unfavorable. Since the highly polar nature of water leads to formidable competitive interactions in this medium, the use of less polar solvents might enable the tetracycline—"X," or oxytetracycline—"X," interactions to assume the position of the principle equilibria with some complexing agents.

In the present study, the complexing tendencies of anhydrous oxytetracycline base and anhydrous tetracycline base, in chloroform and carbon tetrachloride respectively, with N-methyl pyrrolidone, N-N′-dimethyl acetamide, γ-valerolactone, and γ-butyrolactone are presented.

EXPERIMENTAL

Reagents.—N-methyl pyrrolidone, General Aniline and Film Corp., New York, N. Y., Batch 503, redistilled at 95° and 22 mm.; N-N′-dimethyl acetamide, Eastman Kodak "White Label," (4972); γ-valerolactone, Eastman Kodak practical grade, P-6123, redistilled at 206°; γ-butyrolactone, Matheson practical grade, P-7042, redistilled at 206°; Carbon tetrachloride, reagent grade; Chloroform, reagent grade, redistilled before use to remove the ethanol preservative; Ethanol, absolute, analytical grade. Anhydrous oxytetracycline base was prepared from highly purified samples of the base dihydrate by thorough drying *in vacuo* and recrystallization from anhydrous toluene (2). Anhydrous tetracycline base was prepared by purification of the base trihydrate from N-methyl pyrrolidone-water and subsequent thorough drying, *in vacuo*, at 100°.

Procedure.—The solubility characteristics of anhydrous oxytetracycline base required a more polar solvent medium than that of anhydrous tetracycline base. Thus, chloroform was utilized as the solvent medium for interactions involving the former, while carbon tetrachloride was used for interactions involving tetracycline.

Due to a comparatively slow attainment of the solubility equilibria, the interactions were allowed to proceed for forty-eight hours, at 25°, in the constant temperature bath previously referred to (1). In the present study, 5 cc. of standard solutions of the particular complexing agent were added to an excess of either anhydrous oxytetracycline base or anhydrous tetracycline base. Five-cubic centimeter Kimble clear-glass ampuls, with flanged necks, were used as interaction vessels and were sealed before equilibration.

After equilibration, the excess antibiotic was allowed to settle, the ampuls were snapped open, and the liquid filtered through a glass-wool plug (no adsorbtive effect) into a volumetric pipet. The resulting clear solution was suitably diluted, with absolute ethanol, for spectrophotometric measurement (*vide infra*).

The solubilities of anhydrous oxytetracycline and tetracycline in their respective solvents at 25° are as follows: Anhydrous oxytetracycline Base in $CHCl_3$—4.26×10^{-2} moles per liter. Anhydrous tetracycline Base in CCl_4—1.25×10^{-3} moles per liter.

* Received June 4, 1957, from the School of Pharmacy, University of Wisconsin, Madison.

This paper is based in part on a thesis submitted to the Graduate School of the University of Wisconsin by Eugene H. Gans in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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† Present address, Hoffmann-LaRoche, Inc., Roche Park, Nutley 10, N. J.

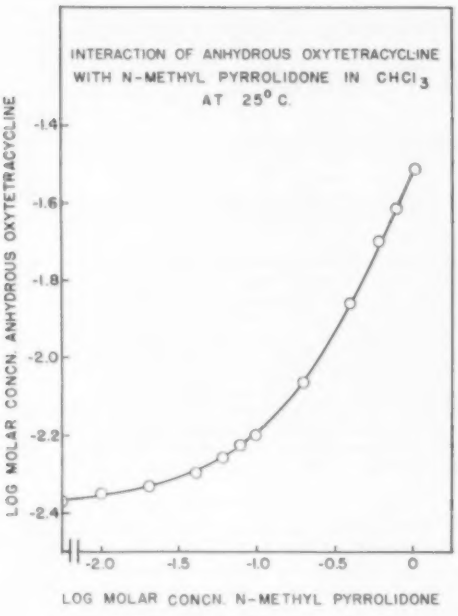
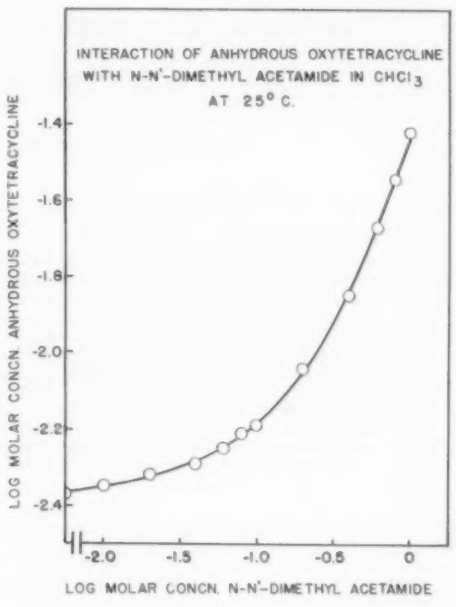


Fig. 1.



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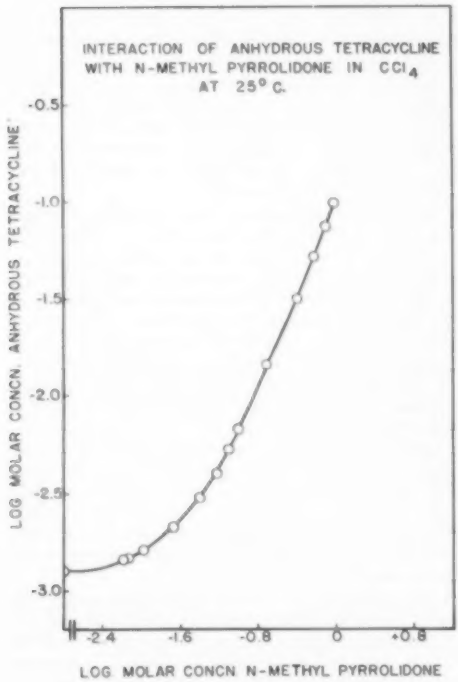

Fig. 2.

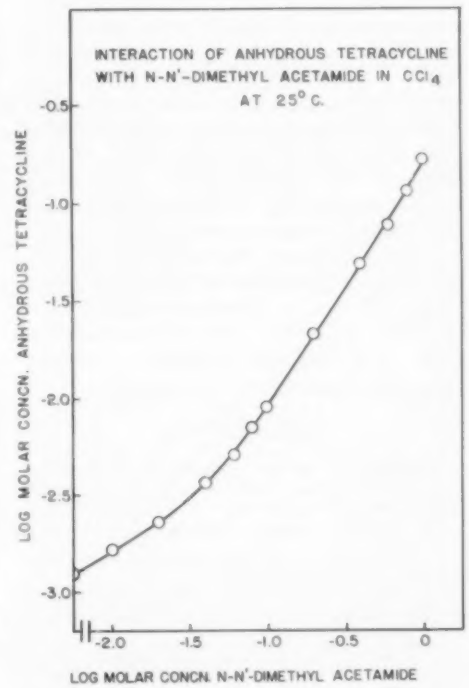

Fig. 4.

RESULTS AND DISCUSSION

N-Methyl Pyrrolidone.—As shown in Fig. 1 for anhydrous oxytetracycline and in Fig. 2 for anhydrous tetracycline, both form soluble complexes, as they did in water (1) with N-methyl pyrrolidone. In these systems, however, the extent of the interactions were considerably greater than those occurring in aqueous solution. In the latter case, at 1.0 *M* N-methyl pyrrolidone, oxytetracycline was solubilized roughly twofold and tetracycline roughly threefold. Herein, at the same concentration of the lactam, anhydrous oxytetracycline in chloroform was solubilized roughly sevenfold, while anhydrous tetracycline in carbon tetrachloride increased its solubility approximately 77 times. Because there is a wide divergence of increase between the two antibiotics in the present systems, undoubtedly due in part to the higher polarity of chloroform as compared to carbon tetrachloride, the effect on the interactions of varying the solvent polarity becomes even more pronounced. While the various differences between the aqueous and nonaqueous systems, and between the two nonaqueous systems, make quantitative comparison difficult, the qualitative aspects show a trend toward increasing interaction with a decrease in the polarity of the mutual solvent.

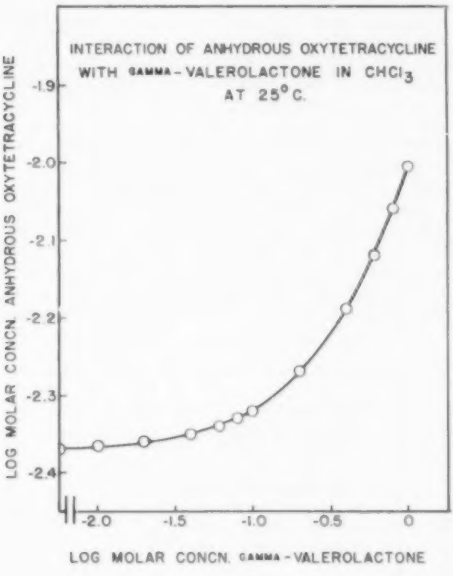

Fig. 5.

N-N′-Dimethyl Acetamide.—The interactions of oxytetracycline and tetracycline with **N-N′**-dimethyl acetamide are shown in Figs. 3 and 4, respectively. The amide appears to have a stronger complexing affinity for the tetracyclines than does N-methyl pyrrolidone. At one molar amide concentration, the solubility of anhydrous oxytetracycline was 3.82×10^{-3} moles per liter, an increase of 9 times the solubility in pure chloroform, while that of anhydrous tetracycline was 1.67×10^{-1} moles per liter, or roughly 133 times that in pure carbon tetrachloride. The solubilizing activity of this simple dimethyl substituted amide is not surprising, since Higuchi and Kostenbauder (3) have shown that dimethyl substituted amides of several dibasic and tribasic aliphatic and aromatic acids exert a pronounced effect on various pharmaceuticals. The

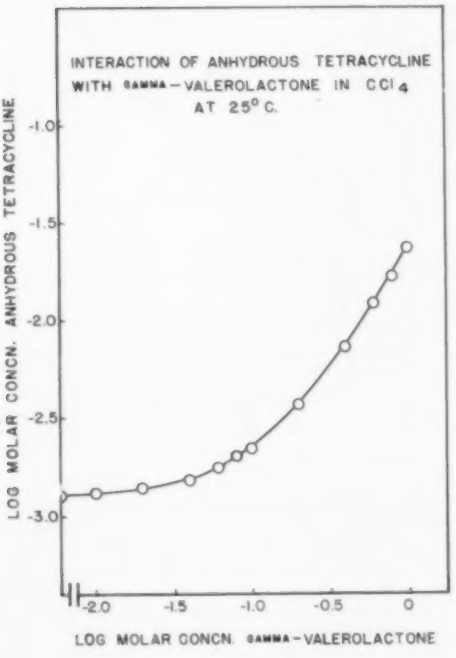

Fig. 6.

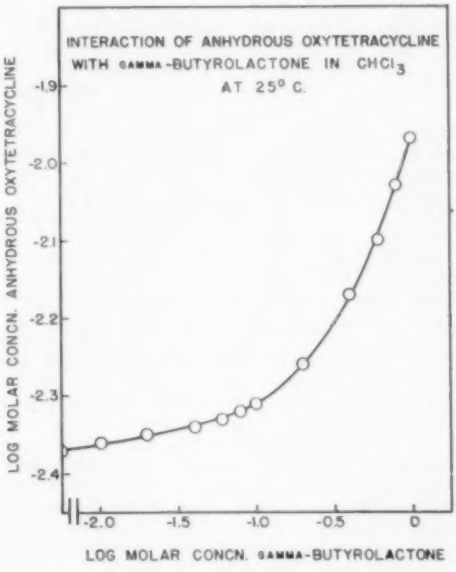

Fig. 7.

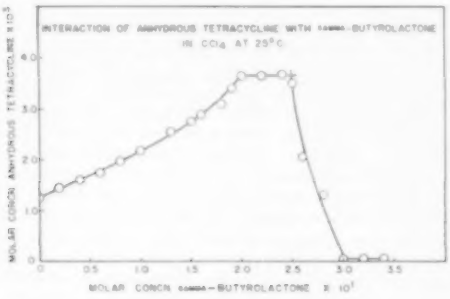
INTERACTION OF ANHYDROUS TETRACYCLINE WITH GAMMA-BUTYROLACTONE IN CCl_4 AT 25°C
MOLAR CONCN. ANHYDROUS TETRACYCLINE X 10^3
MOLAR CONCN. GAMMA-BUTYROLACTONE X 10^1

Fig. 8.

results of their study seem to suggest that the apparent negativity of the carbonyl grouping in dimethyl substituted amides is enhanced by the inductive effect of the methyl groups and/or by the steric hindrance of the methyl groups to the formation of polymeric amide species.

γ-Valerolactone.—The complexing tendency of γ-valerolactone was comparatively weak. As shown in Fig. 5 for anhydrous oxytetracycline and in Fig. 6 for anhydrous tetracycline, the equilibrium concentration, at one molar γ-valerolactone, was 9.89 × 10^{-3} moles per liter for the former and 2.34 × 10^{-2} moles per liter for tetracycline. The respective increases in solubility were twofold and eighteenfold. From the results of the following interactions with γ-butyrolactone, the methyl group of γ-valerolactone appears to play a very important role, perhaps sterically, in the extent to which complex formation takes place.

γ-Butyrolactone.—From Fig. 7 it can be seen that the interaction of γ-butyrolactone with anhydrous oxytetracycline is very similar to that with γ-valerolactone. The solubility of oxytetracycline at one molar lactone is 1.08 × 10^{-2} moles per liter, or roughly two and a half times the basic solubility.

The anhydrous tetracycline-γ-butyrolactone system, shown in Fig. 8, is markedly different. The solubility of the antibiotic initially increases rapidly, as compared to that with γ-valerolactone. However, at a lactone concentration of 2.0 × 10^{-1} moles per liter a plateau is reached and the complex begins to precipitate out of solution. The precipitation continues until a lactone concentration of 2.5 × 10^{-1} moles per liter is reached, at which point the excess tetracycline has been utilized and the solubility curve begins to descend, the soluble complex being transformed into the insoluble form. In many systems of this nature, the solubility curve will level off at the point corresponding to the independent solubility of the solute. However in this system, the solubility curve drops very steeply and for all practical considerations goes to zero concentration of tetracycline at a lactone concentration of 3.0 × 10^{-1} moles per liter.

Isolation of the solid complex, which had a crude crystalline form, and analysis spectrophotometrically and by means of carbon, hydrogen, and nitrogen determinations, showed a stoichiometric relationship of one mole of anhydrous tetracycline to one mole of γ-butyrolactone. The analytical determinations were made on samples produced by interaction of the pure components and by interaction in carbon tetrachloride with, and without, subsequent washing after isolation. In all cases the results corresponded, almost exactly, to a 1:1 ratio. However, consideration of the phase solubility diagram of Fig. 8 with respect to the total amount of tetracycline present will show that other species of a polymeric nature may also be present. The runs were made with 270 mg. of anhydrous tetracycline (mol. wt. 444) per 5 cc., or a concentration equivalent to 1.216 × 10^{-1} moles per liter. During the plateau stage, the amount of tetracycline in solution is equal to 0.0036 moles per liter. Hence, during the plateau interval 0.1216 less 0.0036 moles, or 0.118 moles per liter of anhydrous tetracycline, had undergone complex formation with 0.25 less 0.20, or 0.05 moles per liter of γ-butyrolactone. This corresponds to a stoichiometric ratio of tetracycline 2.36:γ-butyrolactone 1.00. If one considers the interval from the start of the plateau to the point of minimum solubility, equivalent to 0.1 moles of lactone, the ratio decreases to 1.18:1.00. Since the tetracycline is almost completely insolubilized, one might consider that the total tetracycline had been acted upon by the amount of lactone equivalent to the point of minimum solubility, or 0.3 moles of lactone per liter. Then the ratio would be tetracycline 1.00:lactone 2.54 for the overall system. Thus, in conclusion, it can be said that an insoluble 1:1 complex has been isolated and that there is the possibility that other complexes of a polymeric, but weaker, nature might exist. It is also interesting to note the difference between this interaction and

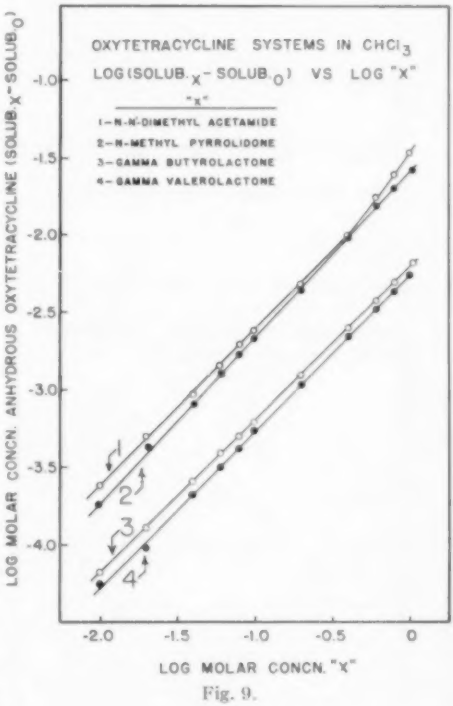

Fig. 9.

the one with γ-valerolactone, the latter differing from γ-butyrolactone by one γ-C-methyl group. Although one might consider the difference to be due to steric factors, the fact that oxytetracycline formed a soluble complex with butyrolactone, while that of tetracycline was ultimately insoluble, does demonstrate the involved nature of these interactions.

Complexing Agent Dependencies.—All the interactions, with the exception of the γ-butyrolactone-tetracycline and the N-N′-dimethyl acetamide-oxytetracycline interactions, displayed the following

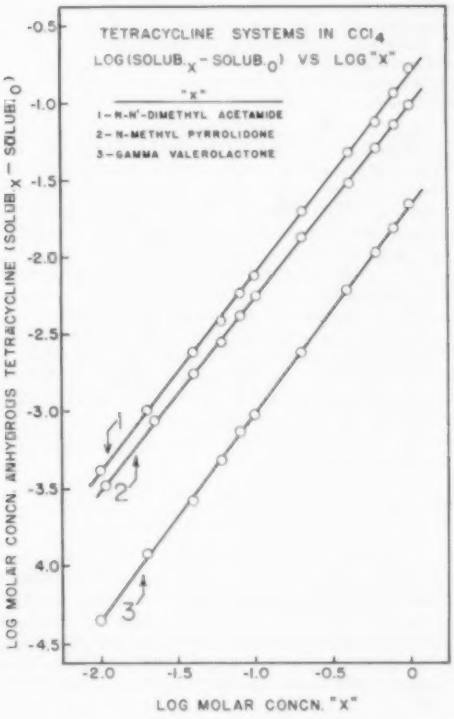

Fig. 10.

linear relationship throughout the concentration range employed: Log $(S_x - S_o)$ = log b + a log "X." The plots for the oxytetracycline systems in chloroform are presented in Fig. 9 and those for the tetracycline systems in carbon tetrachloride are shown in Fig. 10. In the discussion of this exponential relationship in our previous study (1), it was shown that the slope values of the straight line portions of the curves are indicative of the complexing agent dependency. In Fig. 9, for oxytetracycline systems, the initial straight line portion of curve 1 and linear curves 3 and 4 all have a slope of 1.0, while that of number 2 is 1.05. Thus, all the oxytetracycline interactions show a first order dependency and that of N-N′-dimethyl acetamide is seen to increase at higher concentrations of the amide.

The slopes of the linear curves in Fig. 10, for the tetracycline systems, are roughly equal at 1.30 and suggest that these interactions are approximately first order.

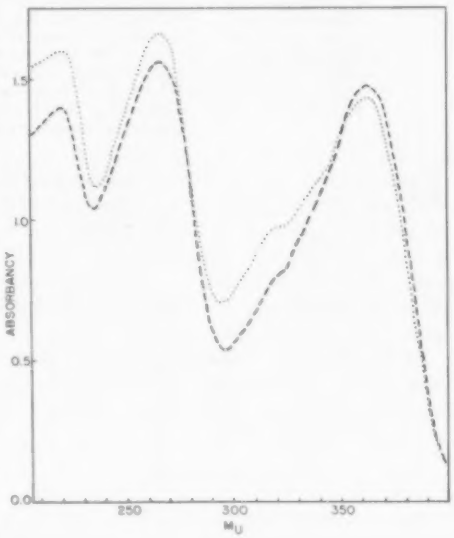

Fig. 11.

Analytical Procedure.—The tetracyclines were analyzed spectrophotometrically, in absolute ethanol, using the Beckman model DU spectrophotometer and/or the Cary model XIV recording spectrophotometer. The ultraviolet spectra of anhydrous oxytetracycline and tetracycline bases in ethanol are shown in Fig. 11. The maxima, in both spectra, at 363.5 mμ was utilized for measurement of the optical density and the determined extinction coefficient values, $E(1\%, 1\text{ cm.})$, in absolute ethanol were: anhydrous oxytetracycline base, 312; anhydrous tetracycline base, 365.

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The Comparative Antispasmodic Activity of N-Methyl-3-Piperidyl Diphenylglycolate Methobromide (JB—340) and Atropine Sulfate*

By J. P. BUCKLEY, J. J. DeFEO, and EDWARD C. REIF

The antispasmodic activity of N-methyl-3-piperidyl diphenylglycolate methobromide (JB-340) was evaluated against atropine sulfate on various segments of the normal and spastic gastrointestinal tract of cats and dogs under pentobarbital sodium anesthesia. JB-340, 0.5 mg./Kg. I.V., exhibited a depressant activity equal to or greater than 0.2 mg./Kg. of atropine sulfate on the colon of cats and dogs. The mean duration of action of JB-340 was approximately equal to atropine sulfate (as determined by blockage of vagal stimuli). Atropine sulfate elicited a spasmogenic effect on the colon of the dog in 60 per cent of the experiments. JB-340, 0.5 mg./Kg. I.V., did not significantly affect the blood pressure of normotensive dogs and did not block the superior cervical ganglion of cats.

DERIVATIVES OF 3-HYDROXY PIPERIDINE have recently been reported to possess powerful anticholinergic activity (1–3). N-Ethyl-3-piperidyl diphenylglycolate methobromide (JB-323) and N-methyl-3-piperidyl diphenylglycolate methobromide (JB-340) demonstrated activity approximately equal to atropine (1).

This present report deals principally with the comparative antispasmodic activity of JB-340 (Cantil) and atropine sulfate on various segments of the gastrointestinal tract.

EXPERIMENTAL

Gastrointestinal Tract in situ.—Cats and dogs of each sex were employed in the present experiments. The animals were anesthetized with pentobarbital sodium (40 mg./Kg. subcutaneously or 35 mg./Kg. intravenously). Balloons, connected to a water-air tambour system or Sanborn electromanometer, were inserted into the lumen of the intestinal segments and stomach by surgical procedures or via the oral and rectal cavities. In all instances the balloons were affixed by ligature. The pressure was adjusted to approximately 20 cm. of water and the activity recorded (4–6). The right vagus was stimulated periodically during the experiments and the effects on the heart and gastrointestinal tract recorded. The stimulus employed was 35 volts at a frequency of 60 per second for a period of ten seconds. The effects of JB-340 and atropine sulfate on the normal and spastic gastrointestinal tract (spasms induced by physostigmine, 0.1 to 0.5 mg./Kg. (7, 8), followed periodically by acetylcholine 50–100 mcg./Kg.) were studied. Minor reference is made to two other piperidyl derivatives *n*-ethyl-3-piperidyl diphenylacetate hydrochloride (JB-305) and JB-323. The responses were graded from +3 to −3 with 0 indicating normal activity, +3 indicating an extreme increase in activity and −3 indicating paralysis. A total of 40 cats and 30 dogs were employed in these experiments.

Superior Cervical Sympathetic Ganglion.—The effect of JB-340 on the superior cervical sympathetic ganglion was studied in four cats anesthetized with pentobarbital sodium. The cervical sympathetic trunk was stimulated periodically with a minimal stimulus using a Harvard stimulator at a frequency of 30 impulses per second and the effect on the nictitating membrane recorded.

Blood Pressure.—The effects of JB-340 on the carotid blood pressure were studied in four dogs anesthetized with pentobarbital sodium. A cannula connected to a Sanborn electromanometer was inserted into a carotid artery and the blood pressure recorded on a visocardiette.

In all experiments the drugs were administered via either femoral vein.

RESULTS

Gastrointestinal Tract in situ.—The spasmolytic activity of the drugs on the normal gastrointestinal motility of dogs and cats will be found in Table I. Table II shows the spasmolytic activity of the drugs on physostigmine-acetylcholine induced spasms of the gastrointestinal tract of dogs and cats. The antispasmodic activity of JB-340 is illustrated in Fig. 1.

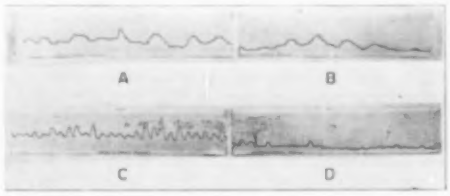

Fig. 1.—The effect of 0.5 mg./Kg. of JB-340 on the intestinal tract of cats. A—Normal colonic motility; B—Colon after JB-340; C—Normal duodenal motility; D—Duodenal motility after JB-340. (Range 0–40 mm. Hg, speed 2.5 mm./sec.)

The anticholinergic activity of 0.5 mg./Kg. of JB-340 was equal to or greater than 0.2 mg./Kg. of atropine sulfate on the colon of the cat and dog. The response of the colon to vagal stimulation was

* Received March 4, 1957, from the Department of Pharmacology, the University of Pittsburgh School of Pharmacy, Pittsburgh, Pa.

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TABLE I.—THE MEAN ANTISPASMODIC ACTIVITY OF JB-340, JB-323 AND ATROPINE SULFATE ON THE GASTROINTESTINAL TRACT OF DOGS AND CATS

Drug	Dose mcg./Kg.	Animal	No. Animals	Duodenum	Stomach	Colon
JB-340	200	D	1	−3.0	...	+3.0
JB-340	200	C	1	−3.0	...	−1.5
JB-340	250	D	1	−3.0	...	−1.0
JB-340	500	D	4	−2.4	−2	−2.5
JB-340	500	C	10	−2.3	...	−1.1
JB-340	1000	C	1	−3	...	−3
JB-323	100	D	2	−2.7	−1.2	−1
JB-323	250	C	1	−3	...	−1
JB-323	500	D	2	−1.5	0	−1
Atropine	100	D	3	0[a]	−2.5	+1.5[a]
Atropine	200	D	5	−2.6	−2	−0.6[b]

[a] Atropine induced duodenal spasms in one preparation and colonic spasms in three preparations.
[b] Atropine induced colonic spasms in two preparations.

TABLE II.—THE MEAN ANTISPASMODIC ACTIVITY OF JB-340, JB-323, JB-305 AND ATROPINE SULFATE ON INDUCED GASTROINTESTINAL SPASMS IN DOGS AND CATS

Drug	Dose mcg./Kg.	Animal	No. Animals	Duodenum		Stomach		Colon		Jejunum	
				S[a]	A[b]	S[a]	A[b]	S[a]	A[b]	S[a]	A[b]
JB-340	500	D	6	...	...	+1.5	−1.3	+2.7	−2.7	...	...
JB-340	32	C	1	+3	−3	...	...	+1	−2	...	...
JB-340	50	C	1	+2.5	−2	...	...	+3	0	+2	−2
JB-340	500	C	9	+2.4	−2.8	...	...	+2.7	−2.2	+3	−3
JB-340	1000	C	2	+2.5	−2.5	...	...	+3	−1.8	+2.5	−3
JB-323	250	C	1	...	...	+2	0	+2	−2	...	...
JB-323	500	C	1	+2	−1.5	...	...	+3	0	...	...
JB-305	1000	C	2	...	...	+1	+0.5	+3	+1	...	..
JB-305	2000	C	1	+3	+1	+3	+1	+3	0	...	...
Atropine	100	D	1	...	...	+3	−2	+3	−2.5[c]	...	...
Atropine	200	D	1	...	...	+2	−3	+3	−3	...	...
Atropine	100	C	2	+3	−2.5	...	...	+2.5	−0.5	...	...
Atropine	200	C	1	+3	−1	...	...	+3	−1	...	...

[a] Effect of spasmogen.
[b] Effect of Antispasmodic.
[c] Atropine induced primary spasms prior to inducing colonic depression.

inhibited by JB-340 for a mean time of one hundred thirty-five minutes and by atropine sulfate for one hundred forty-eight minutes. The mean duration of action of JB-340 on the colon of the cat was five hours (range thirty minutes to eight hours and twelve minutes with 9 experiments out of 13 having a duration of action over four hours). This was approximately 20 per cent longer than the duration of action of atropine sulfate on the colon of the cat.

Atropine elicited a spasmogenic effect on the colonic musculature of the dog in 60% of the preparations, as seen in Figs. 2 and 3, and JB-340 produced this reaction in 16.7 per cent of the dogs to which it was administered. After the initial spasms induced by JB-340, the motility of the colonic musculature was greatly depressed, whereas in most instances the spasms produced by atropine occurred intermittently throughout the experiments and were of much longer duration.

Blood Pressure.—JB-340, 0.5 mg./Kg., produced a transient fall in blood pressure of less than 10 per cent in pentobarbital anesthetized dogs.

Superior Cervical Sympathetic Ganglion.—JB-340, 0.5 mg./Kg., did not block the superior cervical sympathetic ganglion of the cat as the contractions of the nictitating membrane produced by preganglionic stimulation were identical prior to and after administration of the antispasmodic. Small doses of acetylcholine administered intravenously after JB-340 produced maximal contractions of the nictitating membrane.

Other Observed Effects.—JB-340, 0.5 mg./Kg., prevented vagal inhibition of the heart by electrical stimulation of the intact or peripheral end of the severed vagus for a mean time of two hundred twenty-seven minutes (range between one hundred eighty-seven and two hundred eighty-three minutes). Atropine sulfate, 0.2 mg./Kg. prevented the vagal inhibition of the heart in cats and dogs for a mean time of two hundred thirty-three minutes (range between one hundred thirty and three hundred thirty minutes).

JB-340 did not appear to possess demonstrable antisialogogic activity as small doses of acetylcholine produced profuse salivation in all of the animals previously treated with 0.5 mg./Kg. of this anticholinergic compound.

DISCUSSION

The results of these experiments have demonstrated that there is a variation in activity of the anticholinergic compounds on different portions of the gastrointestinal tract. The antispasmodic activity of 0.5 mg./Kg. of JB-340 was greater than 0.2 mg./Kg. of atropine sulfate on the spastic colon of the cat and the normal colon of the dog and equal to atropine on the spastic colon of the dog. The

effects of the upper portion of the gastrointestinal tract appeared to be approximately equal, however the duration of action of JB-340 was usually much less than atropine. From these results it does appear that JB-340 may be an extremely useful anticholinergic agent in controlling hypermotility of the colon.

From the results of these experiments it appears that the principal site of action of JB-340 is at the postganglionic nerve endings of the parasympathetic division and that, at least in those doses administered, it does not possess ganglionic blocking activity.

The spasmogenic property of atropine was demonstrable on the colon in six out of ten dogs. It

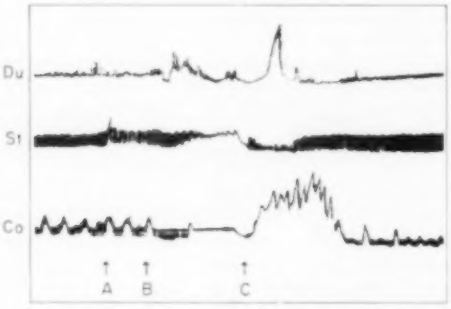


Fig. 2.—Primary spasmogenic activity of atropine in the dog. Du—duodenum; St—stomach; Co—colon; A—Vagal stimulation; B—Atropine sulfate, 100 mcg./Kg.; C—Spasmogenic activity.

cannot be concluded that the stimulation was due to an inactive state of the tissue at the time as the lower bowels were active in five of the six preparations. The stimulatory action appeared to be selective on the colon and did not appreciably affect the upper portions of the gastrointestinal tract. The difference in the antispasmodic activity of atropine in cats and dogs is in agreement with previously published works (9).

SUMMARY

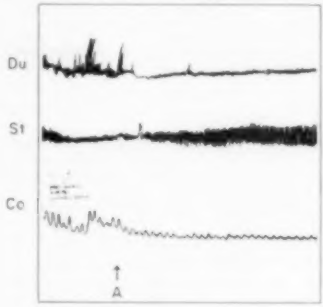
1. N-Methyl-3-piperidyl diphenylglycolate (JB-340) is a powerful anticholinergic compound.

2. The comparative antispasmodic activity of 0.5 mg./Kg. of JB-340 intravenously and 0.2 mg./Kg. of atropine sulfate intravenously is as follows: Spastic colon of the cat—JB-340 > atropine; Normal colon of the dog—JB-340 > atropine; Spastic colon of the dog—JB-340 = atropine; Spastic stomach of the dog—Atropine > JB-340; Normal duodenum of the dog—JB-340 = atropine.

3. JB-340, 0.5 mg./Kg., did not block the superior cervical ganglion.

4. JB-340, 0.5 mg./Kg., appears to have no significant effect on the blood pressure of the normotensive dog.

5. JB-340 does not appear to block significantly the action of acetylcholine on the salivary



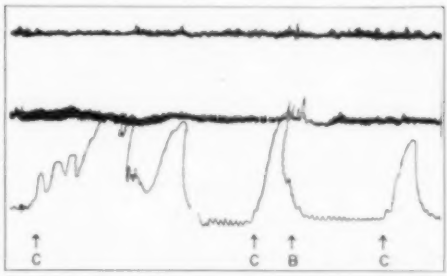


Fig. 3.—Activity of atropine sulfate on the dog gastrointestinal tract. A—Atropine sulfate 100 mcg./Kg.; B—Vagal stimulation; C—Spontaneous colonic spasms.

glands as all of the animals salivated profusely when acetylcholine was administered.

6. The anticholinergic activity of 0.5 mg./Kg., of JB-340 is equal to or greater than 0.2 mg./Kg. of atropine sulfate on the colon of dogs and cats.

7. The duration of action of JB-340 and atropine sulfate are approximately equal.

8. Atropine sulfate produced both a primary and secondary spasmogenic effect on the colonic musculature of dogs.

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An Evaluation of Certain Hypotensive Agents I.*

Diquaternarized Amino Alcohol Esters of Piperidinecarboxylic Acids and a Pyridinecarboxylic Acid

By W. J. HUDAK, J. P. BUCKLEY, F. M. SCHALIT, J. J. DeFEO, and E. C. REIF

A series of eight new bis-quaternized piperidine and pyridine carboxylic acid esters of amino alcohols were screened and evaluated for their hypotensive activity in rats. Further studies of certain of these compounds were conducted on dogs for qualitative correlation. Pentolinium bitartrate and chlorisondamine dimethochloride were used as standards. β-Dimethylaminoethyl N-methyl-α-piperidinecarboxylate dimethobromide was the most promising of the experimental compounds.

MEDICAL SCIENCE is constantly in search of effective hypotensive agents which will lower the blood pressure of the hypertensive patient without interfering with the normal physiological activity and behavior of the individual. At the present time, severe hypertension can best be controlled through the utilization of drugs which reduce blood pressure by blocking nerve transmission through autonomic ganglia. These are the diquaternary ammonium compounds and mecamylamine, a secondary amine.

Burn and Dale (1) observed that tetraethylammonium chloride was capable of blocking the passage of impulses through autonomic ganglia without producing an initial excitatory phase. Hunt (2) confirmed these observations and Acheson (3) further elucidated the activity of T.E.A. on the cardiovascular system. Patton and his colleagues (4–8) reported that hexa- and pentamethylene-bistrimethylammonium compounds interfere with the transmission of impulses at the autonomic ganglia without interfering with the release of acetylcholine.

Pentolinium bitartrate has been reported to be more effective in blocking sympathetic than parasympathetic ganglia thus producing milder side effects (9–11). Chlorisondamine dimethochloride has been reported to be more active than hexamethonium and also to be responsible for fewer side effects (9, 12).

This present report deals principally with the evaluation of diquaternarized amino alcohol esters of piperidinecarboxylic acids and a pyridinecarboxylic acid (Fig. 1). Pentolinium bitartrate[1] and chlorisondamine dimethochloride[2] were utilized as standards.

EXPERIMENTAL PROCEDURES

The Effects of Urethan on the Blood Pressure of Normotensive Rats.—Previous work has demonstrated that urethan exerts only a mild effect on circulation and respiration (13, 14) and is suitable for prolonged anesthesia in a single dose. Since it was imperative that the anesthetic agent not lower the blood pressure of the animals over a prolonged period of time the following experiments were performed prior to the initial screening and evaluation of the experimental compounds. Descendants of Sprague Dawley-Wistar cross rats (four females, six males) were anesthetized with 1.2 Gm./Kg. of urethan intraperitoneally. The left common carotid artery was cannulated in the usual manner using a fine glass cannula connected to a small-bore mercury manometer (3 mm. i. d. glass tubing) by means of rubber and polyethylene tubing. The fluid bridge was 7.5% sodium citrate solution. One-tenth of a milliliter of heparin (1000 U. S. P. units per ml.) was injected directly into the cannula immediately after cannulation. The blood pressure was recorded on a slowly moving kymograph for approximately fourteen hours.

Hypotensive Activity in Normotensive Rats.—Descendants of Sprague Dawley-Wistar cross rats were anesthetized with 1.2 Gm./Kg. of urethan intraperitoneally and prepared as previously described. A minimum of thirty minutes was allowed to elapse to permit stabilization of the blood pressure prior to administering the experimental compounds. Doses of the JB compounds ranging from 2.5 to 200 mg./Kg., and of the controls from 0.5 to 50 mg./Kg., were administered via either femoral vein. The dose of each compound producing a fall in the blood pressure of approximately 50% for a maximum period of time, without obvious signs of toxicity, was selected for further evaluation in a minimum of four rats (equal number of each sex). Chlorisondamine dimethochloride, 0.5 mg./Kg., was administered to 10 normotensive rats and

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This investigation was supported in part by a research grant from Lakeside Laboratories, Inc., Milwaukee, Wis.
The experimental compounds were kindly supplied by Dr. H. L. Daiell, Scientific Director, Lakeside Laboratories, Inc.
Grateful acknowledgment is made to Dr. Joseph Bianculli, Professor of Pharmaceutical Chemistry, for his valuable aid and criticism in preparing this paper.

[1] Generously supplied by Wyeth Laboratories Inc., Philadelphia, Pa., as Ansolysen tartrate.
[2] Generously supplied by Ciba Pharmaceutical Products Inc., Summit, N. J., as Ecolid chloride.

pentolinium bitartrate, 1 mg./Kg., to 11 animals, and the hypotensive activity recorded.

Hypotensive Activity in Normotensive Dogs.—JB-527, 582, 584, and 591 were selected for further studies in dogs. Mongrel dogs of either sex were anesthetized with a mixture of pentobarbital sodium, 30 mg./Kg., and urethan 200 mg./Kg., intravenously or subcutaneously. A vinyl cannula was inserted into the trachea, and the left common carotid artery was cannulated in the usual manner. The system was filled with a 7.5% solution of sodium citrate and the blood pressure was recorded via a mercury manometer or a Sanborn electromanometer. Respiration was recorded using a pneumograph-air tambour system. Electrocardiographic recordings were obtained from lead II in some of the dogs. Fresh solutions of the experimental compounds were administered via the femoral vein.

RESULTS

The Effect of Urethan on the Blood Pressure of Normotensive Rats.—The anesthetic dose of urethan did not lower the blood pressure in any of the prolonged experiments. The average duration of the experiments was 13.8 hours after which time there was a mean rise in the blood pressure of 24% over the initial readings.

Hypotensive Activity in Normotensive Rats.—The hypotensive activity of the experimental and control compounds are summarized in Table I. JB-591 and JB-584 were the most active of the experimental compounds. Although the dosage of these compounds was much greater than that of the controls, the response was more predictable as indicated by the much smaller standard deviations. JB-581 either produced a transient fall or no effect whatsoever on the rat blood pressures. The activity of the remaining compounds was of much shorter duration than the control compounds or JB-591 and 584. Chlorisondamine, 0.5 mg./Kg., was effective for approximately 25% longer than 1 mg./Kg. of pentolinium.

Hypotensive Activity in Normotensive Dogs.—The hypotensive activity of JB-527, 582, 584, and

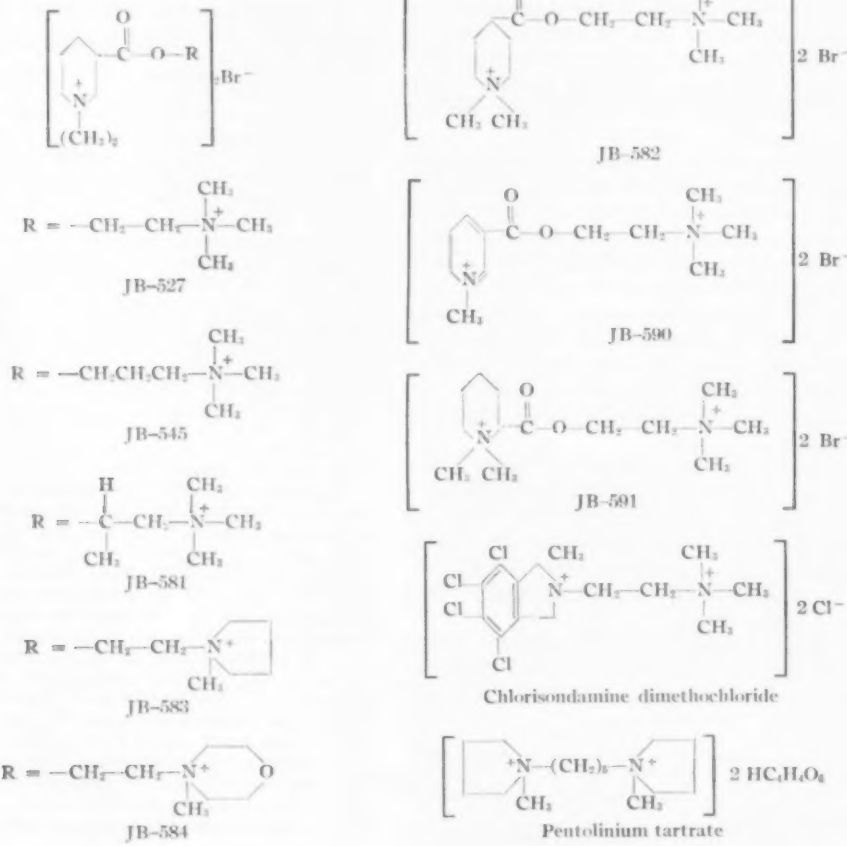

Fig. 1.—Structures of the hypotensive agents investigated.

TABLE I.—THE EVALUATION OF CERTAIN JB-500 AND CONTROL COMPOUNDS ON THE BLOODPRESSURE OF NORMOTENSIVE RATS

Compound	Dose mg./Kg.	No. of Animals	Drop, % ±S. D.	Mean Time to Return to Normal in Min. ±S. D.	Rating[a]
JB-527	20.0	4	55.8 ± 7.8	43.0 ± 12.1	++
JB-545	10.0	4	49.0 ± 10.5	35.0 ± 25.6	+
JB-581	5–100.0	7	...	...	−
JB-582	50.0	4	49.4 ± 10.4	35.8 ± 5.9	++
JB-583	10.0	6	56.7 ± 13.7	47.6 ± 24.0	++
JB-584	10.0	4	56.7 ± 3.7	115.5 ± 18.2	+++
JB-590	50.0	4	52.1 ± 15.5	27.0 ± 10.2	+
JB-591	25.0	4	45.0 ± 9.5	137.2 ± 15.9	++++
Chlorisondamine	0.5	10	46.5 ± 15.1	148.3 ± 58.6	++++
Pentolinium	1.0	11	48.7 ± 18.6	118.5 ± 40.3	+++

[a] Rating: −, not hypotensive; +, fair hypotensive activity; ++, good hypotensive activity; +++, very good hypotensive activity; ++++, excellent hypotensive activity.

TABLE II.—THE EFFECTS OF JB-527, 582, 584, AND 591 ON BLOOD PRESSURE OF NORMOTENSIVE DOGS

Compound	Dose, mg. Kg.	No. of Animals	Drop, %	Time to Return to Normal in Min.
JB-527	20	1	44	159
JB-582	5	1	14	21
JB-582	50	1	18	96
JB-584	10	1	56	288
JB-591	40	5	49	507 [+a]
JB-591	10	1	58	500

[a] Two experiments were terminated with blood pressures 14 and 32 mm. Hg below initial readings.

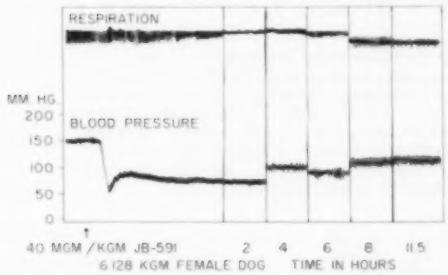

Fig. 2.—The effects of JB-591 on the blood pressure and respiration of a normotensive dog.

591 in normotensive dogs is summarized in Table II (Figs. 2 and 3). JB-591 was the most active of these compounds. The mean time required to return to normal, for a dose of 40 mg./Kg., was over 8.45 hours. Although relatively few dogs were employed with this particular series of compounds, there was a very good qualitative correlation between the results in rats and dogs. JB-591 produced a mild, temporary reversal of the T waves. The electrocardiograms were normal in approximately one hour.

DISCUSSION

The diethylamino derivatives of the series of dimethobromides of dialkylaminoalkyl-N-methyl-β-piperidinecarboxylates exhibited the longest duration of action (JB-527, 583, and 584). The β-morpholinoethyl derivative was the most active of the three compounds. β-Dimethylaminoisopropyl N-methyl-β-piperidinecarboxylate dimethobromide (JB-581) did not demonstrate an appreciable hypotensive activity, whereas the propyl derivative (JB-545) exhibited a fair hypotensive response.

Saturation of the pyridine ring appears to be significant as β-dimethylaminoethyl N-methyl-β-piperidine carboxylate dimethobromide (JB-527), 20 mg./Kg., was more active than 50 mg./Kg. of β-dimethylaminoethyl N-methyl-β-pyridinecarboxylate dimethobromide (JB-590). The α-piperidinecarboxylate derivative (JB-591) had a duration of action approximately three times as long as the β-piperidine carboxylate (JB-527).

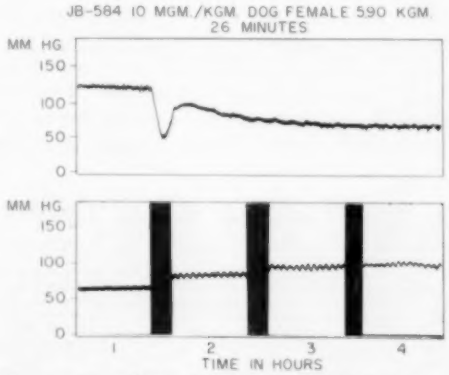

Fig. 3.—The effects of JB-584 on the blood pressure of a normotensive dog.

SUMMARY

1. The relative hypotensive activity of a series of eight new diquaternarized amino alcohol esters of piperidine carboxylic acids and a pyridinecarboxylic acid has been presented.

2. β-Dimethylaminoethyl N-methyl-α-piperidinecarboxylate dimethobromide (JB-591) and β-morpholinoethyl N-methyl-β-piperidinecarboxylate dimethobromide (JB-584) exhibited the longest duration of action.

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An Evaluation of Certain Hypotensive Agents II.*

Acetylenic Bis-Onium Derivatives

By F. M. SCHALIT, J. P. BUCKLEY, W. J. HUDAK, J. J. DeFEO, and E. C. REIF

A series of nine new bis-quaternary and three bis-tertiary ammonium compounds were screened and evaluated for their hypotensive activity in rats. Further studies were conducted on dogs. It was found that there was a definite qualitative correlation between the responses in rats and dogs. Pentolinium bitartrate and chlorisondamine dimethochloride were utilized as standards in the rat evaluation studies. The most promising of the experimental compounds were 1,5-bis(diethylamino)-2-pentyne dimethobromide, 1,6-bis(morpholino)-2-hexyne dimethobromide, and 1,6-bis(pyrrolidino)-3-hexyne dimethobromide.

THE SEARCH for new synthetic hypotensive agents for the treatment of severe hypertension has followed many paths. The most promising and successful to date has been by ganglionic blockade mainly through the use of bis-quaternary ammonium derivatives. This present report deals principally with the evaluation of nine acetylenic bis-quaternary and three acetylenic bis-tertiary ammonium compounds (Fig. 1). Pentolinium bitartrate[1] and chlorisondamine dimethochloride[2] were utilized as standards. The experimental compounds were screened and evaluated for their hypotensive activity only. Data on the pharmacological actions of the most important compounds of this entire series will be presented in a future report.

EXPERIMENTAL PROCEDURES

Hypotensive Activity in Rats.—Descendants of Sprague Dawley-Wistar cross rats were anesthetized with 6 ml./Kg. of a 20% solution of urethan, intraperitoneally. The blood pressure was measured directly from the left common carotid artery, as described in the preceding paper (1). Fresh solutions of the drugs were administered via either femoral vein. The procedures described for obtaining the optimum dose and the evaluation of the compound at these selected dose levels were followed (1).

Hypotensive Activity in Dogs.—JB-520, 537, 540, 562, and 573 were selected for further studies in dogs. Mongrel dogs of either sex were anesthetized with a mixture of pentobarbital sodium, 30 mg./Kg., and urethan, 200 mg./Kg., intravenously or subcutaneously. The animals were prepared as described

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Presented to the Scientific Section A. PH. A., New York meeting, May 1957.
This investigation was supported in part by a research grant from Lakeside Laboratories, Inc., Milwaukee, Wis. The experimental compounds were kindly supplied by Dr. H. L. Daiell, Scientific Director, Lakeside Laboratories, Inc.
Grateful acknowledgment is made to Dr. Joseph Bianculli, Professor of Pharmaceutical Chemistry, for his valuable aid and criticism in preparing this paper.
[1] Generously supplied by Wyeth Laboratories, Inc., Philadelphia, Pa., as Ansolysen tartrate.
[2] Generously supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J., as Ecolid chloride.

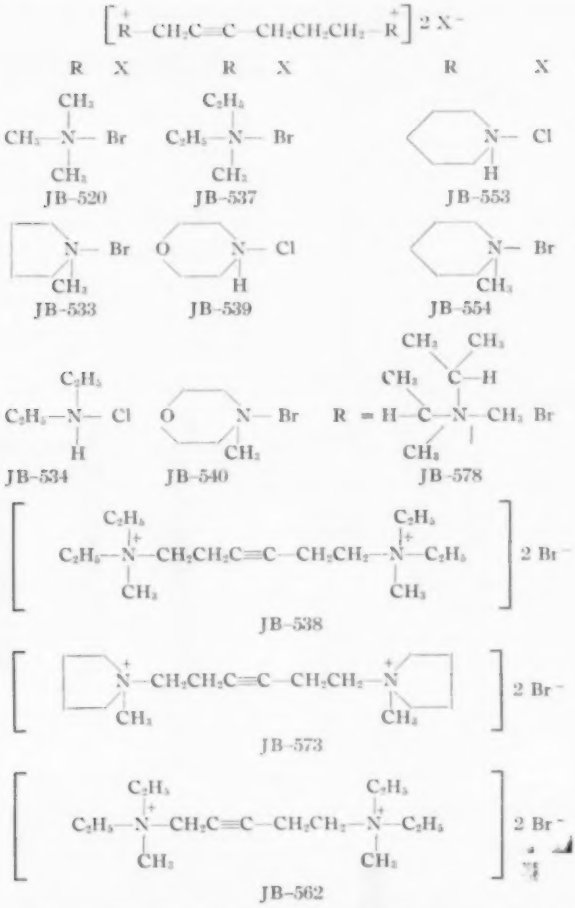

Fig. 1.—Structures of the hypotensive agents investigated.

in our previous report (1). Fresh solutions of the experimental compounds were administered via either femoral vein.

RESULTS

Hypotensive Activity in Rats.—The hypotensive activity of the experimental and control compounds is summarized in Table I. The longest acting of the experimental bis-onium derivatives were JB-562, 540, and 573. The bis-tertiary amines (JB-534, 539, and 553) were relatively inactive in that the dosage required to elicit an approximate 50% drop in blood pressure was very high and the activity was of short duration. One milligram per kilogram of JB-578 produced a mild transient hypotensive response; however, increasing the dosage to 2 mg./Kg., or more, was usually lethal. Although JB-520 was usually active for a fairly long period of time, the duration of action for a given dose was very erratic

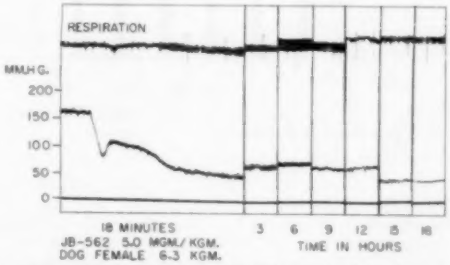

Fig. 2.—The effects of JB-562 on the blood pressure of a normotensive dog.

and in the studies utilizing a 5 mg./Kg. dose one rat out of seven died.

Hypotensive Activity in Dogs.—The hypotensive activity of JB-520, 537, 540, 562, and 573 in dogs is

TABLE I.—THE EVALUATION OF CERTAIN JB-500 AND CONTROL COMPOUNDS ON THE BLOOD PRESSURE OF NORMOTENSIVE RATS

	Dose, mg./Kg.	No. of Animals	Drop, %, ±S. D.	Mean Time to Return to Normal in Min. ± S. D.	Rating[a]
JB-520	5.0	6	48.8 ± 5.9	110.2 ± 98.2	+++
JB-533	2.5	4	59.5 ± 3.0	71.3 ± 19.6	++
JB-534	75.0	4	43.0 ± 2.5	7.0 ± 0.3	+
JB-537	2.5	4	50.8 ± 14.9	78.0 ± 32.6	++
JB-538	1.0	4	45.2 ± 16.3	73.8 ± 45.5	++
JB-539	150.0	4	29.7 ± 9.2	4.1 ± 0.9	+
JB-540	10.0	6	49.3 ± 5.6	165.3 ± 54.8	++++
JB-553	50.0	6	41.0 ± 16.6	7.0 ± 1.7	+
JB-554	5.0	4	53.1 ± 7.2	37.0 ± 22.8	+
JB-562	10.0	4	50.6 ± 3.1	271.5 ±89.3	++++
JB-573	5.0	4	49.9 ± 7.2	132.5 ± 28.7	++++
JB-578	1.0	4	23.5 ± 6.8	3.8 ± 3.5	+
Chlorisondamine	0.5	10	46.5 ± 15.1	148.3 ± 58.6	++++
Pentolinium	1.0	11	48.7 ± 18.6	118.5 ± 40.3	+++

[a] Rating: +, fair hypotensive activity; ++, good hypotensive activity; +++, very good hypotensive activity; ++++, excellent hypotensive activity.

TABLE II.—THE EFFECTS OF JB-520, 537, 540, 562, AND 573 ON THE BLOOD PRESSURE OF NORMOTENSIVE DOGS

Compound	Dose, mg./Kg.	No. of Animals	Drop, %	Time to Return to Normal in Min.
JB-520	1.0	1	15	60
JB-520	5.0	1	18	100
JB-520	20.0	2	44	407
JB-537	2.5	1	44	93
JB-540	1.0	1	34	75
JB-540	2.5	1	28	150
JB-540	10.0	2	51	224
JB-562	0.01	1	...	...
JB-562	0.1	1	16	36
JB-562	0.5	1	22	210
JB-562	5.0	2	66	840+
JB-573	5.0	1	36	177

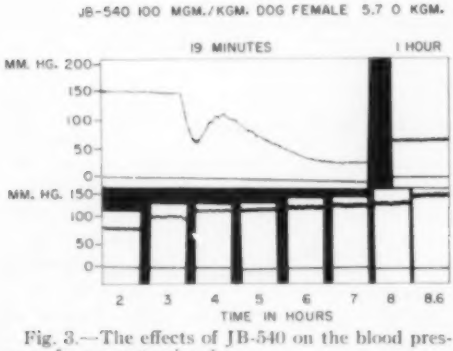

Fig. 3.—The effects of JB-540 on the blood pressure of a normotensive dog.

summarized in Table II. The most active of these compounds was JB-562 (Fig. 2). A dose of 5 mg./Kg. of this compound produced, in two dogs, a mean drop in blood pressure of 66% with a mean time to return to normal of over fourteen hours. Smaller doses of JB-562 elicited milder hypotensive effects for proportionately shorter periods of time. The larger doses of JB-562 markedly affected the respiratory pattern producing a Cheyne-Stokes type of respiration. JB-540 (Fig. 3) and JB-573 were the next most promising compounds, respectively. JB-520 (Fig. 4) produced a prolonged hypotensive effect in dogs when the rat dose was increased fourfold.

One of the most significant observations that can be made at this time is that the results in dogs were almost identical to those in rats. The compounds which proved to be the most active in the rat evaluation studies proved to be the most active drugs in dogs.

DISCUSSION

In the following discussion the compounds were compared on the basis of their respective effective doses and not on a dose-dose relationship.

JB-540, containing a quaternarized morpholino group in the R position was the most active of the six 1,6-bis-quaternary ammonium derivatives of 2-hexyne (JB-520, 533, 537, 540, 544, and 578). It is important to note that the dose of JB-540 was four times that of JB-533 (1,6-bis(pyrrolidino)-2-hexyne dimethobromide) and JB-537 (1,6-bis (diethylamino)-2-hexyne dimethobromide). However, when the latter two derivatives were administered in doses of over 10 mg./Kg., death usually resulted. The bis-tertiary amine derivatives of 2-hexyne were all relatively inactive. A good comparison can be made between JB-539 (1,6-bis(morpholino)-2-hexyne dihydrochloride) and JB-540 (1,6-bis(morpholino)-2-hexyne dimethobromide).

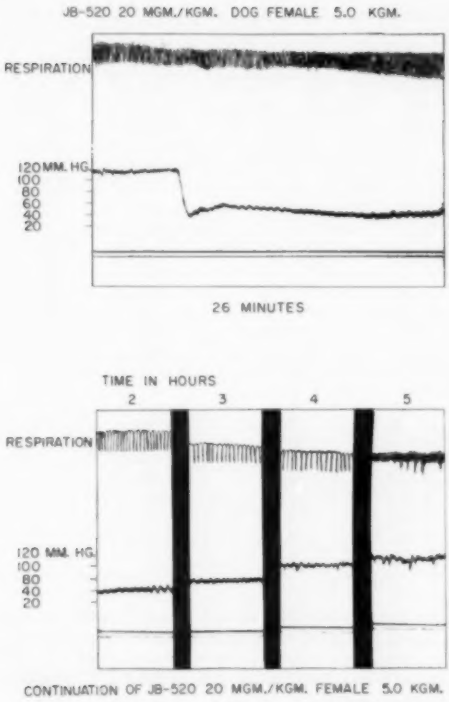

Fig. 4.—The effects of JB-520 on the blood pressure of a normotensive dog.

JB-539, 150 mg./Kg., elicited a mean drop in rat blood pressure of 29.7% with a mean time to return to normal of 4.1 minutes whereas JB-540, 10 mg./Kg., produced a mean drop in blood pressure of 48.8% with a mean time to return to normal of 165.3 minutes.

The bis-quaternarized derivatives of 3-hexyne appeared to be slightly more active than the 2-hexyne derivatives when comparing JB-537 to 538, and JB-533 to 573. JB-562 (1,5-bis(diethylamino)-2-pentyne dimethobromide) was longer acting than the bis-diethylamino derivatives of 2-hexyne or 3-hexyne.

SUMMARY

1. The relative hypotensive activity of a series of nine new acetylenic bis-quaternary ammonium compounds and three acetylenic bis-tertiary ammonium compounds has been presented.

2. 1,5-Bis(diethylamino)-2-pentyne dimethobromide, 1,6-bis(morpholino)-2-hexyne dimethobromide, and 1,6-bis(pyrrolidino)-3-hexyne dimethobromide exhibited the longest duration of action.

3. Data have been presented which validate the use of the direct recording, anesthetized rat preparation in the screening or evaluation of hypotensive agents.

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A Simplified Nitrite Procedure for the Determination of Reserpine*

By DANIEL BANES

A rapid procedure is described for the extraction and determination of reserpine in crystalline samples of the alkaloid and in reserpine tablets.

EARLIER PUBLICATIONS from this laboratory (1) have described a procedure for the colorimetric assay of pharmaceuticals containing reserpine. In that procedure reserpine was separated from more polar alkaloids by a series of extractions with chloroform from dilute acid solution. After careful evaporation of the chloroform, reserpine was determined colorimetrically by a modification of Szalkowski and Mader's nitrite test (2). The simplified method of assay proposed here eliminates both the series of extractions in separators and the evaporation of solvents. Reserpine is separated from more polar alkaloids and from acidic degradation products by elution with chloroform from prepared chromatographic columns. The eluate is then diluted with alcohol, and a fluorescent yellow pigment is formed in the mixed organic solvents by the addition of alcoholic sodium nitrite and acid.

* Received May 29, 1957, from the Division of Pharmaceutical Chemistry, Bureau of Biological and Physical Sciences, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C.

PROPOSED METHOD OF ASSAY

Solutions.—(*a*) Dilute sulfuric acid, approximately half-normal; (*b*) dipotassium hydrogen phosphate (K_2HPO_4), approximately 10% aqueous solution; (*c*) alcoholic sodium nitrite solution,

TABLE I.—ANALYSIS OF CRYSTALLINE RESERPINE SAMPLES

Sample	Source	Reserpine, %
C-1	*R. serpentina*[a]	...
C-2	Unknown	99.7
C-3	*R. vomitoria*	96.9
C-4	*R. vomitoria*	98.1
C-5	*R. serpentina*	98.4
C-6	Unknown	96.3
C-7	Unknown	98.8
C-8	*R. canescens*[b]	97.1
C-9	*R. canescens*[c]	94.6
C-10	*R. canescens*[d]	86.8
C-11	Unknown[e]	93.8
C-12	Unknown[f]	77.7

[a] Used as standard 99.3% by previous method (1).
[b] About 4% deserpidine by chromatography.
[c] More than 5% deserpidine by chromatography.
[d] Deserpidine by chromatography, 14%.
[e] By previous method, 92.0%.
[f] Yellow, impure.

TABLE II.—ANALYSIS OF COMMERCIAL TABLET

Sample	Declared, mg./Tablet	Reserpine, % of the Declared: Proposed Method	Reserpine, % of the Declared: Previous Method
T-1	Simulated, 0.128%	97.7	98.4
T-2	1.0	91.0	91.7
T-3	0.5	80.2	83.0
T-4	1.0	92.9	92.7
T-5	5.0	104.0	100.2
T-6	4.0	95.8	97.2
T-7	0.25	94.8	95.6
T-8	0.25	109.6	111.0
T-9	0.25	91.0	91.2
T-10	0.1	89.6	90.5
T-11	0.25	98.4	99.8
T-12	1.0	99.4	97.4
T-13	0.5	92.5	91.0
T-14	1.0	85.1	83.7
T-15	0.5	95.9	92.3
T-16	0.25	74.0	75.4
T-17	4.0	91.6	
T-18	4.0	86.5	
T-19	5.0	89.6	
T-20	5.0	94.2	
T-21	1.0	97.5	
T-22	1.0	81.2	
T-23	0.25	98.1	
T-24	0.25	80.2	
T-25	1.0	90.9	

0.2%; dissolve about 10 Gm. of sodium nitrite in 100 ml. of water, and store this stock solution in the refrigerator. Mix 1 ml. of the stock solution with 49 ml. of ethanol; (*d*) aqueous sulfamic acid solution approximately 5%, prepared fresh every two or three days.

Preparation of Columns.—The adsorbant, chromatographic tube, packing rod, and technique of packing are described under *Assay* in the U. S. P. XV monograph for Digitoxin (3). Place a small wad of cotton in the tube. Mix 2 Gm. of siliceous earth with 2 ml. of the alkaline phosphate buffer. Transfer to the tube and press down firmly. Cover with about half a gram of water-moistened siliceous earth. Thoroughly mix 2 Gm. of siliceous earth with 2 ml. of dilute sulfuric acid solution, transfer to the tube, and pack down firmly.

Procedure for Crystalline Reserpine.—Protect reserpine and its solutions from strong or direct light. Complete analyses as promptly as possible. Treat the sample and Reference Standard Reserpine identically as follows: Dissolve 25 mg. of reserpine, accurately weighed, in 25 ml. of chloroform.[1] Transfer 5 ml. of the well-mixed solution to the chromatographic column. Wash the column with 5 ml. of chloroform, then elute with chloroform, collecting 49 ml. of eluate in a 50-ml. volumetric flask at a rate of 2–5 ml. per minute. Rinse the tip of the tube, dilute to the mark with chloroform, and mix thoroughly. Transfer 5 ml. of the eluate to a 25-ml. volumetric flask containing 15 ml. of ethanol, and mix by swirling. Dilute to the mark with ethanol and mix.

Transfer duplicate 5-ml. aliquots to separate test tubes. To one of the standard aliquots and to one of the samples add 2.0 ml. of ethanol (the blanks); to the other tubes add 2.0 ml. of alcoholic sodium nitrite solution. Add to each tube five drops of concentrated hydrochloric acid. Shake, and permit to stand for a half-hour. Add 0.5 ml. of sulfamic acid solution and mix. After ten minutes, determine the absorbances of the solutions relative to ethanol at 390 mμ. Calculate the per cent of reserpine in the sample by the formula $[(A - A_0)/(S - S_0)] \times (W_s/W_x) \times 100$, where A and A_0 are the absorbances of the nitrite-treated sample aliquot and its blank, S and S_0 are the corresponding absorbances of the standard aliquots, and W_x and W_s are the weights of the sample and standard, respectively.

Procedure for Tablets.—Analyze a procedural standard as directed under *Crystalline Reserpine*. Weigh a finely powdered sample of tablets equivalent to 5 mg. of reserpine into a 250-ml. beaker. Moisten with 0.5 ml. of ethanol, add 10 ml. of chloroform, cover with a watch glass, and heat to gentle simmering. Boil the mixture carefully for ten minutes with frequent mixing. Cool, add 4 ml. of potassium hydrogen phosphate solution and 6 Gm. of siliceous earth, and mix thoroughly. If the mixture adheres to the beaker add about 1 Gm. of dry siliceous earth, and mix. Transfer quantitatively to a prepared chromatographic column, and proceed as directed under *Crystalline Reserpine*, beginning with the words ". . . elute with chloroform, collecting 49 ml. of eluate. . . ." The quantity of reserpine in the sample weighed, in milligrams, is given by the formula: $[(A - A_0)/(S - S_0)] \times (W_s/5)$ where the symbols are those defined previously.

DISCUSSION

The proposed method of assay was applied to 12 samples of crystalline reserpine from various sources, (C-1 to C-12, Table I), to a known mixture simulating a tablet granulation, (T-1, Table II), and to 24 different manufacturers' lots of reserpine tablets, (T-2 to T-25). The method proved suitable for the analysis of every sample examined. All of the solutions prepared for absorbance measurements were clear and homogeneous.

Analytical values obtained for the samples of crystalline reserpine agreed with the results of analysis by other procedures. Samples C-1 and C-2 in Table I were of the highest purity according to

[1] Aliquots of these solutions also may be used for chromatographic identification tests and spectrophotometric measurements.

both the present method and chromotography; and C-1 was adopted as the reference standard. Assuming a purity of 100% for the standard, recoveries of reserpine from C-1 and C-2 after elution were 98–99%, when compared with unchromatographed standards. The values reported in Tables I and II are based upon procedural standards.[2] Of the twelve samples of crystalline reserpine analyzed, eight contained more than 96% reserpine. Samples C-9 and C-10 contained excessive quantities of deserpidine; samples C-11 and C-12 contained both deserpidine and significant quantities of more polar alkaloids; C-12 yielded the only sample blank with appreciable absorbance at 390 mμ.

Fifteen of the commercial tablet samples were analyzed by both the proposed method of assay and the extended-nitrite procedure previously described (1). Twelve of the check analyses agreed within 2%. The greatest discrepancy between procedures was 4%. Only Sample T-8 contained quantities of 3-dehydroreserpates large enough to give an appreciable blank at 390 mμ. Ultraviolet absorption data for 19 of the 24 prepared chloroform-ethanol solutions indicated that the recovered alkaloidal fractions consisted of reserpine almost exclusively. Paper chromatograms on extracts from the tablets in the most notable of the remaining five samples revealed the presence of other weakly basic alkaloids (rescinnamine and deserpidine).

More polar alkaloids such as reserpic acid, methyl reserpate, ajmaline, and yohimbine are retained on the column quantitatively. Rescinnamine and reserpinine are extracted with reserpine, and yield similar colored derivatives when treated with nitrous acid. But these and other alkaloids can be detected by means of chromatography. The proposed method of assay is as selective as the previously published nitrite procedures (1, 2) and is simpler, quicker, and less vulnerable to manipulative errors.

[2] Solutions of reserpine in ethanol are chromogenically stable for several weeks when protected from light. Since the efficiency of the column is fairly uniform, it is probably necessary to analyze procedural standards only occasionally. Unknowns may be compared with a standard ethanolic solution, diluted with the requisite volumes of chloroform and ethanol just prior to use, and the results corrected for differences between this standard and the procedural standard.

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Antibacterial and Antifungal Properties of β-Naphthol Derivatives IV*

By R. S. BAICHWAL†, MALATI R. BAICHWAL†, and M. L. KHORANA‡

The bacteriostatic and fungistatic activities of a number of β-naphthol and 2-hydroxy-3-naphthoic acid derivatives are reported against *Bacillus typhosus, Micrococcus pyogenes,* var. *aureus,* and *Aspergillus niger*. Selected compounds from the initial screening were further tested for their activity against *Bacillus paratyphosus* A, *Bacillus coli, Bacillus dysenteriae* Shiga, *Micrococcus pyogenes,* var. *albus, Streptococcus-β-haemolyticus, Clostridium tetani, Epidermophyton floccosum, Trichophyton rubrum,* and *Trichophyton gypseum*. Few compounds exhibit activity against Gram-negative organisms at concentrations below 50 μg./cc., and none against *C. tetani*. The bacteriostatic concentration for Gram-positive organisms of 3,6-dibromo-, 3,4′,6-tribromo-, 6-*n*-pentyl-, and 6-*n*-hexyl-β-naphthols and 1,4-dibromo-, 1,6-dibromo-, and 1,4,6-tribromo-2-hydroxy-3-naphthanilides is between 0.2 and 10 μg./cc. Marked inhibition of all the fungous pathogens was shown by 6,6′-dibromo-β-dinaphthol and 1-bromo-β-naphthol. 3-Chloro-, 1-bromo-, 6-bromo-, and 1,6-dinitro-naphthols inhibited the growth of *E. floccosum* and *T. rubrum* at concentrations ranging between 1 and 10 μg./cc.

THE BACTERICIDAL ACTIVITIES of a number of derivatives of β-naphthol have been reported previously (1–3) and the data might be useful in the evaluation of these derivatives as disinfectants and germicides. Much of the information reported on naphthalene and naphthol derivatives relate to their usefulness as insecticides and fungicides against plant pathogens, or as fumigants (4, 5). An extensive investigation of the bacteriostatic and fungistatic properties of β-naphthol derivatives has now been made to select active compounds that could be used as anti-infective agents and to examine the relationship between chemical constitution and anti-infective

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† Present address: Faculty of Pharmacy, University of Toronto, Canada.
‡ Reader in Pharmaceutical Chemistry, Bombay University Department of Chemical Technology, Bombay 19, India.
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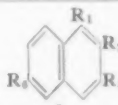
TABLE I.—BACTERIOSTATIC AND FUNGISTATIC ACTIVITY OF β-NAPHTHOL DERIVATIVES[a]



R_1 = H, for compounds 1–23

R_2 = OH, for compounds 24–58

1	2 Compound			3	4	5
No.	R_1	R_2	R_3	*B. typhosus*[b]	*M. pyogenes* var. *aureus*[b]	*A. niger*[c]
			Derivatives of β-naphthol			
1	H	OH	H	100	100	23
2	H	OC_2H_5	H	200[f]	200[f]	18
3	H	$OCH_2CH{=}CH_2$	H	50	100	16
4	H	OCH_2CH_2Cl	H	200	200[f]	19
5	H	$OCOCH_3$	H	200	200	19
6	H	$OCOCH_3$	Br	50	50	14
7	$COCH_3$	OCH_3	H	50	100	15
8	H	OCH_3	$COCH_3$	50	50	14
9	H	OCH_3	CO-*n*Pentyl	50	100	10
10	COC_6H_5	OH	H	50	50	13
11	$COCH_3$	OH	H	100	200[f]	21
12	H	OH	$COCH_3$	50	50	17
13	H	OH	COC_2H_5	200	50	18
14	H	OH	CO-isoPr	200	50	17
15	H	OH	CO-isoBu	200	50	19
16	H	OH	Et	100	10	16
17	H	OH	*n*-Pr	200	10	15
18	H	OH	*n*-Bu	200	10	15
19	H	OH	*n*-Pentyl	100	5	14
20	H	OH	*n*-Hexyl	100	2	10
21	H	OH	isoButyl	200[f]	50	..
22	H	OH	isoPentyl	200[f]	10	..
23	$CH_2C_6H_5$	OH	H	200	10	19
..	R_1	R_2	R_4	...	...	..
24	Cl	H	H	50	50	36
25	Br	H	H	50	50	26
26	I	H	H	200	20	20
27	H	Cl	H	200	100	23
28	H	I	H	20	50	..
29	H	H	Br	50	50	27
30	Cl	H	Cl	100	20	22
31	H	Cl	Cl[d]	100	20	28
32	Br	H	Br	50	10	20
33	Br	Br	H	200	100	22
34	H	Br	Br	50	5	16
35	Cl	Cl	Cl[d]	200	50	19
36	Br	Br	Br	100	5	10
37	Br[d]	Br	Br	20	5	19
38	Cl	H	Br	50	10	20
39	I	H	Br	50	20	20
40	Cl	Cl	Br	200	20	15
41	H	OH	H	200	200	20
42	NO	H	H	50	100	18
43	NO_2	H	NO_2	200	200	25
44	1′ — $C_{10}H_6$-OH(β)	H	H	200	20	17
45	1′ — $C_{10}H_5OH$-βBr6′	H	Br	50	5	26
			Derivatives of 2-hydroxy-3-naphthoic acid			
46	H	$COOCH_3$	H	50	200[f]	13
47	H	$COOC_2H_5$	H	50	200[f]	14
48	H	COOnPr	H	50	200[f]	13
49	H	$COOC_6H_5$	H	50	200	10
50	H	$COOC_6H_5$	$OCOCH_3$[e]	200	200[f]	17
51	H	COO-*p*-Cl-*m*-cresyl	H	50	200[f]	11
52	H	COOH	Br	200[f]	200	18
53	Br	$CONHC_6H_5$	H	200[f]	5	10
54	H	$CONHC_6H_4Br(4')$	H	200[f]	20	10
55	Br	$CONHC_6H_4Br(4')$	H	200[f]	0.5	10
56	Br	$CONHC_6H_5$	Br	200[f]	0.5	10
57	Cl	$CONHC_6H_4Cl(4')$	Cl	20[f]	10	..
58	Br	$CONHC_6H_4Br(4')$	Br	200[f]	0.2	..

See footnotes page 605 →

TABLE II.—INHIBITORY CONCENTRATIONS IN μg./cc. OF SELECTED COMPOUNDS[a] AGAINST GRAM-NEGATIVE ORGANISMS

Compound[b]	*B. typhosus*	*B. paratyphosus A*	*B. dysenteriæ* Shiga	*B. coli*
24	50	50[c]	50	50[c]
28	20	50	50[c]	50[c]
29	50	50[c]	50	50[c]
32	50	50[c]	50	50[c]
37	20	50[c]	50	50[c]
39	50	50[c]	50	50[c]
40	50	50	50	50[c]
46	50	50	50	50[c]
47	50	50	50[c]	50[c]
49	50	50	50[c]	50[c]

[a] Showing inhibition at 50 μg./cc. against Gram-negative organisms other than *B. typhosus;* Cultures obtained from Haffkine Institute, Bombay.
[b] Numbers refer to compounds included in Table I.
[c] More than.

properties. Preliminary *in vitro* screening of 126 derivatives was carried out and the selected compounds tested against several other Gram-positive and Gram-negative bacteria as well as common dermatophytes. Detailed studies on the effective compounds will be published subsequently.

EXPERIMENTAL

Synthesis.—Some of the compounds were available commercially, but for the majority of them synthetic methods reported in the literature were employed. Methods of synthesis are recorded in previous publications (1–3, 6–8).

Bacteriostatic Tests.—The broth dilution method using *B. typhosus* (Lister) and *M. pyogenes* var. *aureus* (FDA 209) was followed. Concentrations of 200,

TABLE III.—INHIBITORY CONCENTRATIONS IN μG./CC. OF SELECTED COMPOUNDS AGAINST GRAM-POSITIVE ORGANISMS

Compound[a]	*Strept.-β-hemolyticus*[b]	*M. pyogenes* var. *aureus*[c] (FDA 209)	*M. pyogenes*[c] var. *aureus*[c] (Virulent)	*M. pyogenes* var. *albus*	*C. tetani*[d]
19	5	5	5	5	50[d]
20	5	2	2	5	50
34	10	5	5	5	50
36	20	5	20	10	...
37	10	5	5	5	50[d]
45	10	5	5	10	...
53	50	5	20	10	...
55	2	0.5	2	1	50[d]
56	2	0.5	2	1	50
58	1	0.2	0.5	0.5	50

[a] Numbers refer to compounds included in Table I.
[b] Isolated from patients at Nair Hospital, Bombay.
[c] Showing inhibition at least at 5 μg./cc. against *M. pyogenes* var. *aureus* (FDA 209); Cultures obtained from Haffkine Institute, Bombay.
[d] More than.

TABLE IV.—FUNGISTATIC ACTIVITY OF SELECTED COMPOUNDS[a] AGAINST DERMATOPHYTES

	Agar Streak Method[c]			Agar Cup-Plate Method[d]		
Compound[b]	*E. floccosum*	*T. rubrum*	*T. gypseum*	*E. floccosum*	*T. rubrum*	*T. gypseum*
1	50	50	50	16.5	22.5	20.5
25	10	10	50	48	47	28
27	10	10	50	18.5	20	19
29	10	10	50	19.5	31	26
30	50	10	50	16	23.5	15.5
31	50	50	50	17	21	16
32	10	10	50	11.5	18	13
37	50	50	50	15	17.5	13
38	10	10	50	13	21	15
39	50	1	50	20.5	23	18
42	50	50	50	13	24	18
43	10	10	50	16	24	20.5
45	50	10	50	33.5	36.5	28

[a] Showing inhibition of all the fungous pathogens at least at 50 μg./cc.; Cultures obtained from A.F.M.C. Poona, India.
[b] Numbers refer to compounds included in Table I.
[c] Inhibitory concentration in μg./cc.
[d] Mean diameter of zone of complete inhibition in mm. at 0.25% concentration with a cup of 10mm. diameter.

[a] Compounds inhibiting growth of *B. typhosus* and/or *M. pyogenes* var. *aureus* at least at 50 μg./cc., and/or exhibiting 16 mm. or more clearance zone against *A. niger*.
[b] Inhibitory concentration in μg./cc.
[c] Mean diameter of zone of complete inhibition in mm. at 0.25% concentration with a cup of 10mm. diameter.
[d] Substituent in 4 position.
[e] Substituent in 2 position.
[f] More than; ...not tested.

100, and 50 μg./cc. were prepared by the addition of an alcoholic stock solution, and further dilutions such as 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 μg./cc. were made where necessary. The tubes were incubated for forty-eight to seventy-two hours following inoculation with a loopful of a twenty-four-hour broth culture of the test organism. Controls indicated the absence of bacteriostasis by the concentrations of alcohol used. Compounds which inhibited the growth of the organisms at 50 μg./cc. or at lower concentration are recorded in Table I.

Further evaluation of the compounds inhibitory at 50 μg./cc. was made using *B. paratyphosus* A, *B. coli*, *B. dysenteriæ*, Shiga, *M. pyogenes* var. *aureus* (virulent), *M. pyogenes* var. *albus*, *Strept. β-hæmolyticus* and *C. tetani*. The results are recorded in Tables II and III.

Fungistatic Tests.—Ten cubic centimeters of Sabouraud's agar containing one cc. of a standardized spore suspension of *A. niger* (NRRL 337) were poured over 20 cc. of agar and the plates incubated for six hours (9). Alcoholic solutions of concentration 1, 0.5 and 0.25% w/v were then added to the prepared cups and plates incubated for forty-eight to seventy-two hours at 25–27°. The mean diameters of the zones of complete inhibition for the concentration of 0.25% are reported in Table I.

Selected compounds showing activity against *A. niger*, were further tested against *E. floccosum*, *T. rubrum* and *T. gypseum* by the agar-streak (10) and the agar cup-plate (9) methods. In the streak method, streaks of spore suspension were drawn over agar plates containing the test substances in concentration of 200, 50, 10, and 1 μg./cc.. Control plates containing alcohol exhibited no inhibition. Results obtained after incubation for five to six days by both the methods are recorded in Table IV.

DISCUSSION

The relationship between chemical structure and bactericidal activity has been discussed previously (1–3). Some of the observations on the data presented here appear similar.

Blocking of the hydroxyl group depresses the bacteriostatic as well as fungistatic activity of the parent compound or its halogenated derivative. Introduction of a keto group slightly enhances the bacteriostatic activity, but increasing the chain length in the 6-substituted ketones does not further enhance the activity.

Alkylation increases the bacteriostatic activity, while halogenation influences the fungistatic activity as well. With an increase in the chain-length or the number of halogen atoms the activity is increased especially against *M. pyogenes* var. *aureus*. However, the position of substitution has a marked influence. Compared to the straight-chain compounds, iso-alkyl derivatives were inferior; but halogenated tertiary-alkyl-β-naphthols have been recently reported (11) to be "staphylostatic" at high dilutions. Methylation of alkyl-naphthols is detrimental as is the case with substitution of different halogen atoms in the parent naphthol.

Groups such as hydroxy, nitro, amino, and sulphonic do not enhance the anti-infective power of β-naphthol. 1:6-Dinitro-β-naphthol, however, exhibits marked inhibition of *A. niger*. Of the β-dinaphthols studied, β-dinaphthol and its dibromo derivative appear to be superior to the corresponding compounds in the β-naphthol series.

The alkyl and aryl esters of 2-hydroxy-3-naphthoic acid show preferential activity against *B. typhosus* which is not enhanced with increasing chain-length. The iso-alkyl esters or their halogenated derivatives are also inactive. Modification of the carboxyl group to an amide, anilide or hydrazide, or halogenation of the acid and its amide does not augment the activity. However, bromoanilides inhibit the growth of *M. pyogenes* var. *aureus* at very low concentration; but this depends markedly on the position occupied by the halogen atom.

Most of the selected compounds (Table II) are active against the Gram-negative organisms, excepting *B. coli*, at 50 μg./cc. but they are effective at 20 μg./cc. The inhibitory concentrations of all the compounds in Table III against the two strains of *M. pyogenes* var. *aureus* tested, do not differ significantly. Although *M. pyogenes* var. *albus* and *Strept. hemolyticus* require a higher concentration for inhibition, the specificity of activity shown by these compounds is noteworthy.

Results of fungistatic activity (Table IV) indicate that halogenation of β-naphthol enhances the activity but polyhalogenation or substitution of different halogen atoms fails to enhance it further. Although some derivatives exhibit fungistasis at 10 μg./cc. against *E. floccosum* and *T. rubrum* by the streak method, the activity against each organism by the cup-plate method differs from compound to compound and no generalizations can be made.

SUMMARY

A bacteriostatic and fungistatic examination has been made of 126 compounds related to β-naphthol and 2-hydroxy-3-naphthoic acid using *B. typhosus*, *M. pyogenes* var. *aureus*, and *A. niger;* and some conclusions on the relationship of chemical constitution and anti-infective properties are drawn.

Whereas alkylation of β-naphthol enhances the bacteriostatic activity, halogenation increases both bacteriostatic and fungistatic activity. However the position of substitution and a free phenolic group are influencing factors.

Esters of 2-hydroxy-3-naphthoic acid show preferential activity against Gram-negative organisms. Bromoanilides, depending on the position occupied by the bromine atom, inhibit the growth of Gram-positive organisms at low concentrations.

Selected compounds were further evaluated using the organisms *B. paratyphosus* A, *B. coli*, *B. dysenteriæ*, *M. pyogenes* var. *albus*, *Strept. hemolyticus*, *C. tetani*, *E. floccosum*, *T. rubrum*, and *T. gypseum*. For Gram-positive organisms, the inhibitory concentrations of 3,6-dibromo-, 3,4,6-tribromo-, 6-*n*-pentyl-, and 6-*n*-hexyl-β-naphthols and 1,4′-dibromo-1,6-dibromo-, and 1,4′,6-tribromo-2-hydroxy-3-naphthanilides lie between 0.2 and 10 μg./cc. Results of the cup-plate method indicated 1-bromonaphthol and 6,6′-dibromo-β-dinaphthol as the most active compounds against the dermatophytes tested. Fungistasis at 10 μg./cc. against *E. floccosum* and *T. rubrum*, by the streak method was exhibited by five halogenated derivatives and 1,6-dinitro-β-naphthol.

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Solution Rate of Theophylline Salts and Effects from Oral Administration*

By EINO NELSON

The solution rate of several theophylline salts was determined in buffer solutions covering a range of hydrogen ion concentrations somewhat greater than those to which the salts would be exposed after oral administration. It was found that marked differences existed in the rate with which the several salts dissolved and the rates were relatively independent of buffer pH. It was suggested that the fundamental reason for differences found by clinical workers in comparing blood levels from oral administration of the choline and isopropanolamine salts with the ethylenediamine salt was due to differences in solution rate. The *in vitro* solution rates determined strongly support this explanation. The effect of solution rate on maximum blood level and rate of build-up of blood level with time was discussed by means of a mathematical model relating blood level with solution rate and elimination rate from the blood stream. The solution rate of theophylline in acidic and basic media was determined and found to vary inversely with the hydrogen ion concentration of the diffusion layer. A relationship was developed between solution rate of theophylline and diffusion layer pH and it was shown to be capable of roughly predicting solution rate of salts from measurement of this pH.

A NUMBER OF SALTS of the weak acid theophylline have been made available commercially and several are currently used in therapeutics. Part of the advantage claimed to be gained in the use of one particular salt in preference to another or the free acid itself is enhanced water solubility (1, 2). It is difficult to see how this factor could be of importance when the drug is administered orally. In the first place, the theophylline contained in the usual dose of most salts (0.2 Gm.) is soluble in 25–30 ml. of gastric fluid and in a slightly smaller volume of intestinal fluid. In the second place, the small amount of base introduced when any of the salts are taken could not possibly raise the pH of either gastric or intestinal fluid high enough to increase the solubility of theophylline in these fluids.

In spite of the foregoing argument, there is convincing clinical evidence available to show that different blood levels of theophylline were obtained when several salts were studied comparatively. In one study, markedly higher levels were obtained from the choline salt when compared to the levels from the ethylenediamine salt (1). In another, oral administration of the isopropanolamine salt resulted in higher average levels than those from the ethylenediamine salt (2). The results obtained were not explainable on the basis of theophylline content of the preparations tested.

This work was conducted to determine the *rate* with which several salts of theophylline dissolved in mediums possessing hydrogen ion concentrations in the physiological range since it was thought that this factor could logically explain the clinical results reported (1, 2). A supporting purpose of this work was to consider the mathematics of the *solution* → *absorption* → *elimination* processes after oral administration of

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drugs in the solid state to the extent of formulating an expression to relate maximum blood level and rate of build-up of blood concentration with solution rate. Additional studies were made on the solution rate of theophylline itself in acidic and basic media and on other topics related to the main problem.

THEORY

The expression generally accepted to describe the rate of the solution process is known as the Noyes-Whitney Law (3). This expression gives rate as a function of the several variables controlling the process as follows:

$$da/dt = KS(C_s - C) \qquad \text{(Eq. 1)}$$

where a is the amount dissolved, t is time, S is the surface area of the dissolving solid and K is the solution rate constant which is a function of the diffusion coefficient of the dissolving specie, the rate of agitation of the medium and other factors. The quantity C is the concentration in the dissolution medium and C_s the concentration of dissolving solid at the liquid-solid interface. According to theory, a thin film of liquid saturated with the dissolving solid is always present at this interface. This film is known as the diffusion layer and, other things being constant, the rate of solution is controlled by diffusion of material through it.

It was thought that the properties of the diffusion layer would be of primary importance in determining the rate at which several freely soluble basic salts of the slightly soluble, weak acid theophylline would dissolve. If the diffusion layer were saturated with dissolving solid even when the dissolution medium was acidic, then the rate of dissolution of the salts would be rapid compared to the rate at which the free acid would dissolve. The rate for the salts would be determined mainly by the pH of the diffusion layer, other things being constant. The rate for dissolution of the free acid would also depend on the pH of the diffusion layer but this should be mainly controlled by the pH of the medium whereas the properties of the salts themselves would determine pH (in the diffusion layer).

The experimental arrangement in this work was such as to allow use of Eq. 1 in the following differential form:

$$da/dt = KSC_s \qquad \text{(Eq. 2)}$$

i.e., C was always negligible in comparison to C_s. Integrating Eq. 2 gives

$$a = KSC_st \qquad \text{(Eq. 3)}$$

Equation 3 should also give the amount of material dissolved *in vivo* from a constant surface since absorption after solution would keep C small in relation to C_s.

EXPERIMENTAL

Materials and equipment.—Blue M constant temperature bath, model MW 1152; Sigmamotor pump, model T-6; Sargent synchronous rotator, 500 r. p. m.; Dissolution assembly (see text); Beckman pH meter, model G; Beckman spectrophotometer, model DU with 1-cm. silica cells; Carver laboratory press, 10 ton; Perkin-Elmer evacuable KBr die, model 021-0106; Cenco, Hyvac vacuum pump; Time-it, Precision Scientific Co; Theophylline and salts (see below); and A. R. grade reagents.

Preparation of Samples.—Rate studies were made using cylindrical pellets of theophylline and several of its salts,[1] 1.3 cm. in diameter and about 0.4 cm. thick, made from −200 mesh powder. The purity of the salts was determined by the spectrophotometric method of Comer and Hilty (4) (see Table I). The pellets were made by compression at 100,000 psi in an evacuable punch and die assembly. Prior to compression, air pressure in the die and powder sample was reduced to 2–4 mm. mercury. The die wall and punch faces were painted with a slurry of magnesium stearate in ethyl alcohol before charging.

The pellets prepared as described above were mounted in the center of 1 x 3 inch microscope slides with molten paraffin. Only the upper face of the pellet was left free of paraffin.

Dissolution Assembly.—The pellets prepared and mounted as above were placed in the bottom of a 600-ml. beaker which had four projections facing toward the interior, so arranged that a slide with pellet would not rotate when dissolution medium was placed into the beaker and stirred. Movement of the slide was prevented also by placing a lead ring of appropriate diameter and coated with paraffin in the bottom and on the slide in the beaker.

The 600-ml. beaker was immersed in a constant temperature bath and held there by a metal framework and clamps. It was immersed to a depth sufficient to cause about 90% of its peripheral surface to be surrounded by bath water.

Dissolution medium contained in the beaker described was arranged to be stirred by a paraffin-coated, stainless steel, paddle-type stirrer (1 x 3 cm.) driven by a 500 r. p. m. constant speed motor. The medium was arranged to be sampled by means of a Sigmamotor pump which cycled the media during the solution rate tests at the rate of about 250 ml per minute. The return from the pump was arranged so that it could easily be manually withdrawn and periodically positioned over test tubes to draw samples for analysis.

Procedure for Solution Rate Determination.—After fixing the dissolution beaker in its mounting in the constant temperature bath and installing the slide bearing the test specimen in its bottom, the stirrer was positioned so that it was in the center and its blade immersed to a depth of 4 cm. with dissolution medium in the beaker. The bath water and 500-ml. dissolution medium contained in graduate were brought to 25°. The stirrer and pump were started, and medium poured rapidly into the beaker. At this time an electric timer was started. At twenty- or forty-second intervals the pump return was removed from the dissolution beaker and positioned over test tubes to withdraw 25-ml. samples for analysis. Immediately after sample withdrawal, fresh medium was added to replace that withdrawn. Three to five withdrawals were made per test.

The samples collected as described above were

[1] The salts were very graciously supplied by the following organizations: choline, Nepera Chemical Co.; ethanolamine, Eli Lilly and Co.; isobutanolamine, Wm. S. Merrill Company; isopropanolamine, National Drug Co.

analyzed for theophylline by the spectrophotometric method of Comer and Hilty (4). This analysis was at 270 millimicrons using 0.1 *N* HCl as the solvent and blank. No correction was necessary for the small amount of buffer solution in some of the solution analyzed. Absorbance values were expressed as grams theophylline per 500 ml. by the absorbance equivalent: 10 mcg./ml. = 0.545 absorbance units. The concentrations determined for the second, third, fourth, and fifth samples withdrawn were corrected for the amount of theophylline lost from the dissolution medium by previous withdrawals. This correction is summarized by the following expression: $C_n = C_n^\circ + V_w/V \times C_{n-1}$ where C_n is the corrected concentration for the nth withdrawal, C_n° the concentration actually determined and C_{n-1} the corrected concentration of sample withdrawn immediately before the nth and V_w and V, respectively, the volume in withdrawal and volume of dissolution medium

A portion of the last sample collected from each run was taken for pH measurement. All tests, determinations and pH measurements were made at 25°.

Validity of the Experimental Method.—The method of pellet preparations adopted was to insure that each salt tested presented the same surface to the dissolving medium. At the pressure of compression used, and particularly when compressions were made *in vacuo*, all pelleted salts could be assumed to be at their true density (5). The surface roughness of each of the pellets could not have been greater than the diameter of the largest particle in the powder sample compressed. If the diffusion layer between solid and medium was thicker than the distance between the highest and lowest points on the pelleted solid's surface, then the dissolving medium in all cases would "see" a constant surface. The thickness of the diffusion layer may be calculated from an equation discussed by Jost (6):

$$l = \sqrt{nl/vd} \qquad \text{(Eq. 4)}$$

where l is thickness in cm., n viscosity of the medium in poise, d the density of the medium, l the linear dimension of surface of the solid in cm. across which the medium flows and v the velocity of the flow of medium in cm./sec.

In the tests reported here, the medium could be considered rotating as a block over the surface of a circular pellet with center place at its axis of rotation. At the middle of any thin annular section of a test disk at radius $r + dr/2$ from the center, the linear velocity of the medium was equal to $\omega(r + dr/2)$ where ω was the angular velocity of the medium in radians/second. At this same radius, the linear dimension of the surface was $2\pi(r + dr/2)$. Substituting for v and l and using the identity, ω is equal to 2π revolutions per second (r. p. s.), in Eq. 4 gives

$$l = \sqrt{n/d(\text{r. p. s.})} \qquad \text{(Eq. 5)}$$

Now, since any annular section could have been taken with distance $r + dr/2$ from center of disk and since dr may be as small as desired, the diffusion layer was of constant thickness at all points on the surface of the pellet providing turbulent flow did not occur.

For the media used, the ratio of n/d was never less than 0.01 and since 500 r. p. m. was the rotation speed of the stirrer, the thickness of the diffusion layer was found to be no less than 0.035 cm. Since −200 mesh particles are about 0.0075 cm. in diameter, the diffusion layer easily covered any irregularities in the surface of the test pellets. Actually, while the movement of the medium was quite regular, its velocity was much less than stirring speed, due to drag on the container wall and bottom; so the diffusion layer was much thicker than indicated by this calculation. The appearance of the disks after tests also indicated that the flow of the mediums were quite regular.

Scope of Experimental Determination.—The dissolution rates for all materials listed in Table I were determined in 0.1 *N* HCl. In addition, the dissolution rates of theophylline and the ethylenediamine, isopropanolamine, and choline salts were determined in buffers consisting of 0.1 *M* disodium phosphate and 0.1 *M* monobasic potassium phosphate with a pH of 6.70 and 0.1 *M* sodium borate and 0.1 *M* boric acid with a pH of 8.87. The three mediums covered a range of pH's somewhat greater than those to which orally administered drugs would usually be exposed. In addition, the solution rate of theophylline was determined in media covering a range from 0.1 *N* to 0.2 *N* strong base. Concurrently with the rate studies, determinations were made of the pH at 25° of saturated solutions of theophylline and all of the salts used in the different dissolution media to obtain values for the pH of the diffusion layers. The water solubility of the salts were determined after equilibration at 25° in a constant temperature bath fitted with a rotating, spoked wheel which held vials containing excess solute and solvent.

RESULTS AND DISCUSSION

Solution Rate in 0.1 *N* HCl.—Figures 1 and 2 show the rate behavior of the several salts and theophylline in 0.1 *N* HCl. These plots are corrected to a common time base. This was desirable since a definite time was usually needed to reach equilibrium in the diffusion layer as evidenced by finding a small positive time intercept when plotting concentration *vs.* time data from studies with the salts and a negative time intercept plotting the same data from determinations with theophylline. This means that the salts initially dissolved at a slower rate than the

TABLE I.—PURITY OF COMPOUNDS USED IN SOLUTION RATE STUDIES

Compound	Theophylline, %: Theoretical	By Analysis[a] 1	2	Av.
Theophylline, U. S. P.	90.9	91.8	90.8	91.3
Choline Theophyllinate	64.4	63.2	63.1	63.2
Ethanolamine Theophyllinate	74.7	74.4	74.2	74.3
Ethylenediamine Theophyllinate U. S. P.	78.9	82.8	82.0	82.4
Isobutanolamine Theophyllinate	67.0	68.9	68.6	68.8
Isopropanolamine Theophyllinate	70.7	70.1	70.9	70.5

[a] By the method of Comer and Hilty (4).

TABLE II.—SUMMARY OF SOLUTION RATE PER UNIT AREA DATA FOR THEOPHYLLINE SALTS IN SEVERAL MEDIA

Salt	0.1 *N* HCl		0.1 *M* Phosphate Buffer		0.1 *M* Borate Buffer	
	Rate[a]	D.L. pH[b]	Rate	D.L. pH	Rate	D.L. pH
Choline	21.1	11.75	12.3	11.09	13.2	11.30
Ethanolamine	6.32	9.45	...	...	...	...
Ethylenediamine	4.68	9.38	4.77	9.27	4.37	9.27
Isobutanolamine	6.26	9.75	...	...	...	...
Isopropanolamine	13.3	9.67	15.0	9.64	14.3	9.60

[a] Gm./Sq. cm.-sec. × 10^4.
[b] Diffusion layer pH.

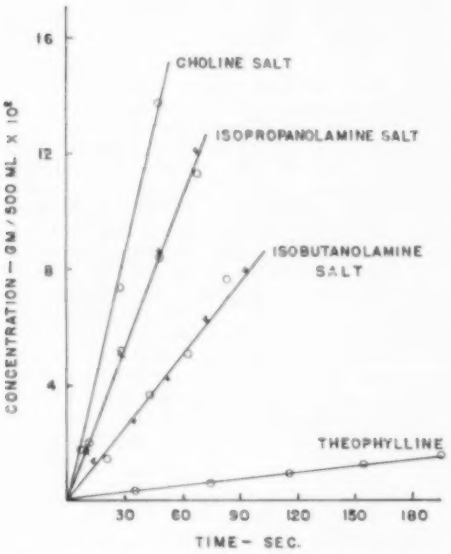

Fig. 1.—Solution rate of theophylline and salts in 0.1 *N* HCl.

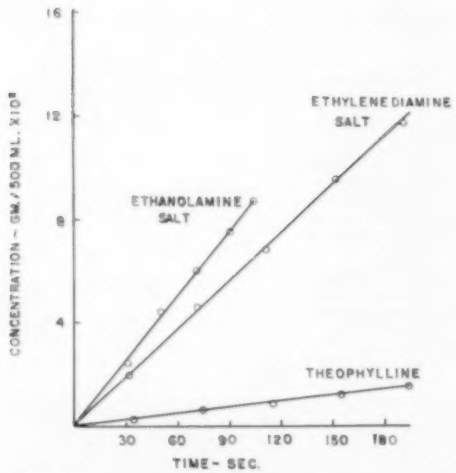

Fig. 2.—Solution rate of theophylline and salts in 0.1 *N* HCl.

steady state reached and theophylline at an initially faster rate than the steady state. Other factors possibly causing positive or negative time intercepts were uncertainty in zero time and the possible presence of a small amount of lubricant on the face of the pellets from the compression process. The correction was never greater than the time in a withdrawal period during tests.

Figures 1 and 2, and Table II clearly show that the postulated condition in the diffusion layer must exist. Table II has been prepared from the slopes of the curves of the figures to show the approximate correlation between solution rate and diffusion layer pH. It might be thought that the relationship between rate and diffusion layer hydrogen ion concentration should be given by the following equation which was derived from Eq. 2 by replacing the C_s term with an expression relating the solubility of a weak acid and hydrogen ion concentration (7, 8):

$$da/dt = KS\,\{[HA] + [HA]\,Ka/[H^+]\} \quad \text{(Eq. 6)}$$

where $[HA]$ is the molar solubility of the free acid. This expression only approximates the rates. The deviations could occur for several reasons. The cation as well as the anion must diffuse from the diffusion layer since electroneutrality must be maintained there. Since the cations are of different molecular weights and probably possess different degrees

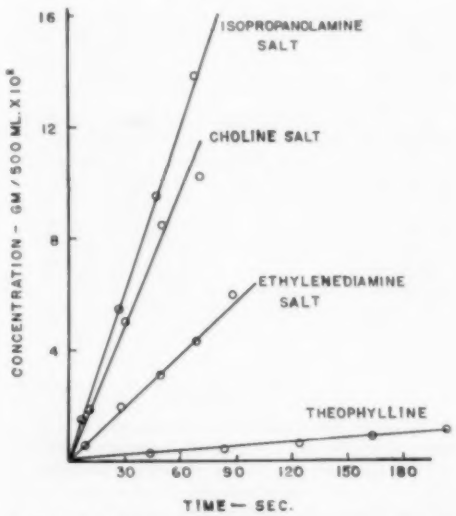

Fig. 3.—Solution rate of theophylline and salts in 0.1 *M* phosphate buffer.

of hydration, the diffusion rate of each could not be expected to be the same. The saturated solutions of all salts were much more viscous than the media. This would affect the diffusion rate of both the anion and cation and also increase the thickness of the diffusion layer.

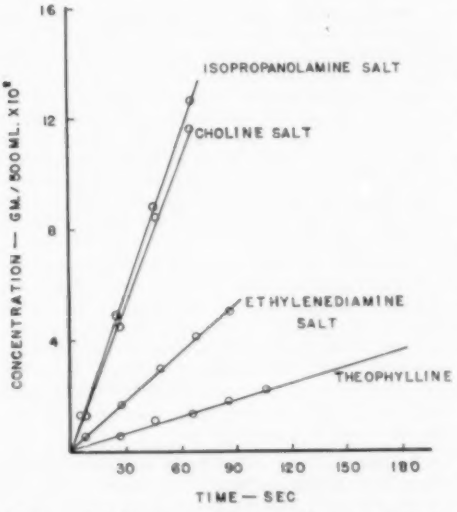

Fig. 4.—Solution rate of theophylline and salts in 0.1 *M* borate buffer.

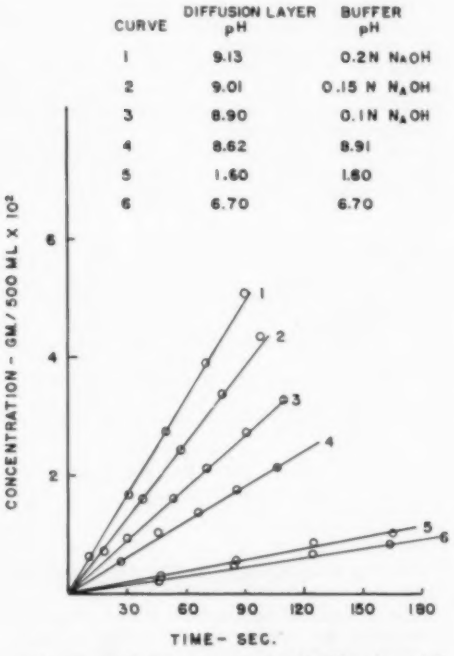

Fig. 5.—Solution rate of theophylline in acidic and basic media. Buffer and diffusion layer pH's indicated in table at top.

No measurable change occurred in the pH of any medium in the above tests. This would be expected since only small amounts of the solids were dissolved during tests.

Solution Rate in 0.1 *M* Phosphate and 0.1 *M* Borate Buffer.—The rate behavior of theophylline and several salts in 0.1 *M* phosphate buffer and 0.1 *M* borate buffer is shown in Figs. 3 and 4. The variation in rate was as expected from information gained in the studies in acid media. The rate of solution per unit area of the pellets derived from the slopes of the curves of Figs. 3 and 4 also compared with diffusion layer pH's in Table II. The lack of precise correlation between these quantities is no doubt due to the factors discussed in the preceding section. The pH of these buffers were unchanged by the tests.

Solution Rate of Theophylline.—The results obtained from determination of the solution rate of theophylline in acidic and basic media are shown in Fig. 5. The progressive increase in rate with increasing basicity was expected. The curve for the determination in 0.1 *N* HCl lies above that for buffer of pH 6.7 since theophylline has a basic function with $K_b = 1.9 \times 10^{-14}$ (9). In pH's above those at which significant amounts of cation form the rate of solution should follow Eq. 6. Plotting rate per unit area, as determined from the curves of Fig. 6 *versus* the quantity, $(1 + K_a/[H^+])$ where K_a is the acidic dissociation constant of theophylline at 25°, 1.69×10^{-9} (9), and $[H^+]$ was determined

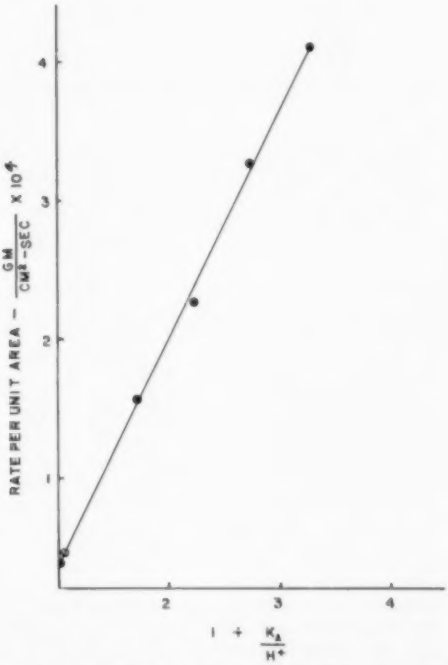

Fig. 6.—Relationship between solution rate of theophylline and hydrogen ion concentration of diffusion layer. See text for explanation of open circle on line.

from measurement of the pH of the diffusion layer yields the graph of Fig. 6. The agreement between theory and experiment is surprisingly good. Accordingly, the solution rate of theophylline at the stirring rate used in these experiments is given by the following expression:

$$da/dt = 1.07\,S(1 + K_a/[H^+]) \quad \text{(Eq. 7)}$$

where $1.07 = K/[HA]$. The ordinate of the open point on Fig. 7 was determined from the slope

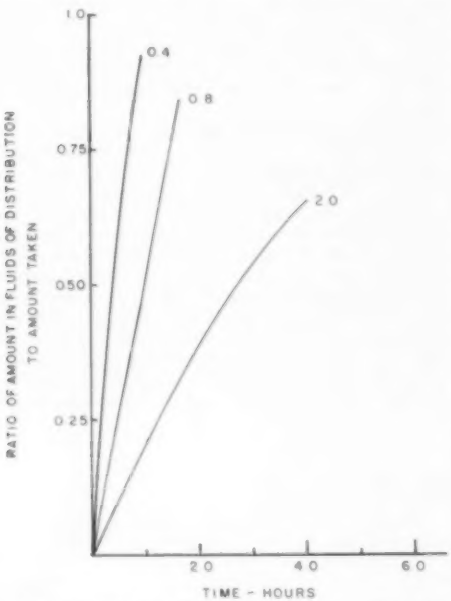

Fig. 7.—Effect of solution rate on amount of drug in fluids of distribution at various times. Curves marked with values of half-solution times (ρ). Curves end at $t = 2\rho$ at which time all drug is dissolved and maximum level reached.

of the curve of Fig. 5 for dissolution in 0.1 *N* HCl and the abscissa value was calculated from $(1 + K_b/[OH])$ since the corresponding theoretical relationship for a weak base should be: $da/dt = KS([B] + K_b[B]/[OH^-])$ where $[B]$ is the molar solubility of the free base.

The rate of solution for the various salts in the media used can be roughly estimated from Eq. 7 and measurement of the pH of the solutions taken to represent the diffusion layer. The calculated and measured results are compared in Table III. The deviations are due to the same effects mentioned in discussing the dissolution of the salts in acidic and basic media.

Water Solubility and Solution Rate.—There appeared to be only poor correlation between water solubility and solution rate of the salts used in the tests. Water solubility and solution rate in 0.1 *N* HCl are compared for all the salts used in Table IV. In the particular compounds used here, water solubility indirectly plays a part in determining solution rate but by itself is an unacceptable explanation for results obtained in clinical trials, as previously discussed. Water solubility is dependent on the strength and water solubility of the base from which the salt was formed.

The water solubility of the salts of theophylline given in Table IV followed the expected relationship between this quantity and hydrogen ion concentration of the saturated solution. It was apparent, however, that the ionic strength of the saturated solutions was high enough to prevent calculation of solubility in the pH range 9.0 to 10.0 using literature values for the acidic dissociation constant and solubility of the free acid (9).

Relation Between Blood Level and Solution Rate After Oral Administration.—From results presented in the preceding sections, it is clear that in a range of pH's likely to be encountered by theophylline salts after oral administration, differences may exist in the rate with which several will dissolve. Further, in the clinical work previously mentioned, which in one case was a comparison between the choline and the ethylenediamine salts (1), and in the other between the isopropanolamine and ethylenediamine salts (2), the choline and isopropanolamine salts both showed higher and more prolonged levels than the ethylenediamine salt. In the studies here reported, both salts dissolved between three and five times as fast as the ethylenediamine salt depending on the medium. This strongly suggests that rate of solution is a logical explanation for the clinical results.

The influence of solution rate is made even more convincing by considering the following mathematical treatment of the absorption process after oral administration. Beginning with solid drug at absorption sites, say in the small intestine, the

Table III.—Comparison between Calculated and Measured Solution Rates of Theophylline Salts

Salt	Rate[a] Found	Rate[a] Calcd.	Diffusion layer pH	Buffer Medium
Ethylenediamine	4.68	5.91	9.38	0.1 *N* HCl-pH = 1.6
Isopropanolamine	13.3	9.52	9.67	0.1 *N* HCl-pH = 1.6
Ethylenediamine	4.77	4.37	9.27	0.1 *M* Phosphate pH = 6.7
Isopropanolamine	15.0	8.94	9.64	0.1 *M* Phosphate pH = 6.7
Ethylenediamine	4.46	4.37	9.27	0.1 *M* Borate pH = 8.91
Isopropanolamine	14.3	8.24	9.60	0.1 *M* Borate pH = 8.91
Ethanolamine	6.32	5.08		0.1 *N* HCl-pH = 1.6
Isobutanolamine	6.26	10.15		0.1 *N* HCl-pH = 1.6

[a] Gm./Sq. cm.-sec. × 10^4.

processes by which the drug is absorbed and eliminated may be depicted by the following scheme:

$$A \rightarrow B \rightarrow C \rightarrow D$$

Here A represents solid drug at the absorption sites, B drug in solution at the absorption sites, C drug in the blood and other fluids of distribution, and D degraded drug. The rate of the transition of A to B is the solution process which is dependent on the several variables previously discussed. The rate of transition, B to C, is not known for the drugs studied here, but can be assumed to be concentration dependent in such a manner as to cause an increase in rate with increase in concentration. The rate of elimination of drug from the blood stream, transition C to D, is known in the case of theophylline to be by a first order process with a half-life of 2.9 hours (10).

Since the rate of the absorption step B to C is not known, assume that the solution step, A to B, is slow enough to make it the rate determining step of the two. There is evidence that this is the case with aspirin (11). The possible effects of this assumption on the development to follow will be discussed later. Rewriting the scheme shown in accordance with this assumption as

$$A \rightarrow B \rightarrow C$$

where B is now drug in fluids of distribution and C is degraded drug, results in a process that can be treated mathematically with known functional relationships in each transition. The amount of drug in the blood at any time, B_t, will be the difference between the amount initially present, B_i, plus amount that entered during the same time, B_{in}, minus the amount that left, B_o. Expressed mathematically, this statement becomes Eq. 8.

$$B_t = B_i + B_{in} - B_o \qquad \text{(Eq. 8)}$$

Taking the derivative of Eq. 8 with respect to time gives

$$dB_t/dt = (dB_{in}/dt) - (dB_o/dt) \qquad \text{(Eq. 9)}$$

When elimination is by a first order process, the rate out, dB_o/dt, will be equal to the product of the specific elimination constant, K_d, and B_t, since $dB_o/dt = dC/dt = K_dB_t$ where dC/dt is the rate of formation of degradation product or elimination. Therefore,

$$dB_t/dt = (dB_{in}/dt) - K_dB_t \qquad \text{(Eq. 10)}$$

Now, assume the salts were administered as a thin disk, or a number of such disks, whose diameters were large in respect to thickness, as was the case with the test specimens used in these experiments. If this were the case, the surface of the dosage form would remain essentially constant during the dissolution process after administration. Then Eq. 2 could be written to describe the rate of solution or availability for absorption as

$$da/dt = K' \qquad \text{(Eq. 11)}$$

where K' includes all right-hand terms of Eq. 2 and the number of small discs administered. Now, dB_{in}/dt in Eq. 10 is equal to da/dt. Substituting in Eq. 10, gives

$$dB_t/dt = K' - K_dB_t \qquad \text{(Eq. 12)}$$

which is easily integrated and constant evaluated to give an expression relating the ratio of the amount of drug in the fluids of distribution to the amount initially administered during the time solid drug remains at the absorption sites. Hence,

$$B_t/A_o = 0.732d/p\,(1 - e^{-0.693t/d}) \qquad \text{(Eq. 13)}$$

In Eq. 13, the constants K_d and K' have been replaced by, respectively, the half-life of the drug in the blood stream, $d = 0.693/K_d$ and half-solution time, $p = 0.5A_0/K'$. A_o is the amount of drug initially administered. Equation 13 holds between $t = o$ and $t = 2p$ at which time all drug is dissolved at the absorption site. After $t = 2p$, the blood level would be described by a first order decay process.

Taking 2.9 hours as the half-life of theophylline in the blood stream (10) and assigning 0.4, 0.8, and 2.0 hours to represent estimated *in vivo* half-solution times of the choline, isopropanolamine, and ethylenediamine salts, respectively, the plot of Fig. 7 has been made. The difference in half-solution times taken was approximately of the same order as the difference rates in the solution rates studied. From Fig. 7, the effect of solution rate is easily seen since the maximum level and rate of build-up of blood level are markedly affected by the value of this quantity. If the derivative of Eq. 13 is taken with respect to time, it will be seen that rate of build-up varies inversely with half-solution time.

An equation similar to Eq. 13 may be developed for the drug in any initial solid state of aggregation. However, the resulting expressions become quite complex, but the considerations as determined from Eq. 13 will hold. Eq. 13 and Fig. 7 are not intended to predict blood levels, but only to show the influence of solution rate on this factor.

If the absorption process is not rapid when compared to the solution rate process, the inferences

TABLE IV.—COMPARISON BETWEEN WATER SOLUBILITY OF THEOPHYLLINE SALTS AND THEIR SOLUTION RATE IN 0.1 *N* HCl

Salt	Solubility[a, c] grams/liter	Solubility[b, c] grams theophylline/liter	pH of saturated soln.	Rate[d]
Choline	729	470	12.31	21.1
Monethanolamine	291	217	9.58	6.32
Isobutanolamine	531	356	9.76	6.26
Isopropanolamine	509	360	9.78	13.3
Ethylenediamine	190	150	9.45	4.68

[a] Calculated on basis of theoretical composition.
[b] Analysis by method of Comer and Hilty (4).
[c] At 25°.
[d] Gm./Sq. cm.-sec. × 10^4.

drawn from Eq. 13 will hold as long as absorption rate increases with increase in concentration. It seems unlikely that the contrary would be true in view of Cori's work on absorption of organic acids (12). The effect of delay due to slow absorption would be to necessitate a more complicated term for the quantity $dB_{(n)}/dt$ in Eq. 9.

Comments on Absorption of Orally Administered Drugs.—The *in vitro* results found here should also be found for salts of any poorly soluble, weak acid with weak and strong water soluble bases. The higher blood levels obtained from the potassium salt, for example, when comparing the benzathine and potassium salts of benzyl penicillin (13, 14) should be explainable on the same basis as the work reported here, since the potassium salt may be expected to dissolve much faster. Sodium pentobarbital should act more rapidly and give a higher blood level than the free acid because it will dissolve faster after being taken. The sodium salt of aspirin taken in solution will act more rapidly than the acid in a tablet, since time will not be needed for the solution step. Many more examples could be cited.

In comparative clinical testing of orally administered drugs, the investigators seem concerned only with absorption of the drug in different forms. There is a surprising lack of awareness among these investigators concerning the effect the solution step will have on their findings. There is an awareness among a few pharmaceutical investigators of the importance of this factor (11, 15).

The solution step should also be considered in toxicity determinations from orally administered drugs since maximum level will depend on this factor.

The dosage form of the drug can influence or override the solution rate properties of a drug. A tablet containing a fast dissolving drug would not gain full benefit from this property if it were compressed so hard that its disintegration time was excessively long.

Two sets of capsules each containing identical amounts of the same drug could on the average give different blood levels if the state of dispersion of the contents of capsules in one set differed from the other set. This is, of course, a consequence of Eq. 1 which states that rate varies directly with surface. Two salts of the same drug compared clinically for relative blood levels after oral administration could give different average levels if they both had the same intrinsic dissolution rate for the same reason. In fact, an intrinsically slower dissolving salt could be made to give higher average levels than those obtained from an intrinsically faster dissolving salt by modifying the surface area that the latter presents to the dissolving medium.

SUMMARY

A marked difference was found to exist in the rate with which several salts of theophylline dissolved in mediums with hydrogen ion concentrations in the range to which the drugs would be exposed after oral administration. The differences in rates were related to the pH of the diffusion layer which covers the surface of dissolving solids and were relatively independent of medium pH. The rates were roughly predictable from measurement of the diffusion layer pH and the solution rate constant for theophylline determined from studies on its rate of solution in basic media. By a simplified mathematical model it was shown that, with other factors remaining constant, solution rate determines maximum blood level and rate of build-up of blood level with time. Clinical results previously reported on comparative blood levels from several salts of theophylline were explained here as being due to differences in solution rate.

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Study of Complex Formation by Polyethylene Glycol and Dimethylurea with Some Pharmaceuticals*

By J. L. LACH, K. RAVEL, and S. M. BLAUG

The formation of molecular complexes in aqueous solution by the interaction of pharmaceuticals and some complexing agents has been reported. However, the complexing agents used in these studies were high molecular weight compounds such as polyethylene glycol, carboxymethylcellulose, polyvinylpyrrolidone, and the xanthines. Experimental evidence is offered to support the existence of the formation of complexes in solution between sym-dimethylurea, a highly soluble low molecular weight compound, and *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-aminosalicylic acid, and acetylsalicylic acid. Phenobarbital did not undergo interaction with sym-dimethylurea. The interaction of polyethylene glycol and the parabens was also studied. The effect of such interaction on the antibacterial activity of the parabens is currently under investigation.

THE FORMATION of molecular complexes in aqueous solution by the interaction of pharmaceuticals and some complexing agents has been reported (1–5). However, the complexing agents used in these studies were high molecular weight compounds such as PEG, carboxymethylcellulose, PVP, and the xanthines. *sym*-Dimethylurea, a highly soluble, low molecular weight compound, was investigated for purposes of comparison; since, on the basis of a previously proposed theory a carbonyl group of an amide, being a good electron donor, would be expected to show an attraction for the electron-accepting groups of compounds having acidic hydrogen.

Additional information on the interaction of PEG and certain pharmaceuticals, such as the parabens and PABA, would be of value in studies being conducted in these laboratories on the effect of this reaction on the antibacterial activity of the compounds under investigation.

EXPERIMENTAL

Interactions with sym-Dimethylurea.—In this study the solubility method previously described (4) has been employed to determine the extent of the complexing tendency of polyethylene glycol 4000 and 6000 and dimethylurea with some pharmaceutical compounds.

Data from these solubility studies are recorded in Fig. 1. The phase diagram shows the effect of varying concentrations of dimethylurea on the solubility in water at 30° of the compounds investigated. It is evident that these acidic compounds, with the exception of phenobarbital, do undergo molecular complex formation with dimethylurea as shown by their increase in solubility. The lack of interaction of phenobarbital is attributed in part to a greater competition of dimethylurea for the water molecules than for the phenobarbital molecules.

* Received January 7, 1957, from the State University of Iowa, College of Pharmacy, Iowa City.

These molecular reactions may be of the type: Dimethylurea + $_n$Acid $\rightleftharpoons$ Dimethylurea – Acid$_n$. No attempt was made to calculate the stability constants for these reactions, since an exact stoichiometrical relationship of amide-acid ratio concentration of the complex is required. The solubility method employed in these studies does not permit such a calculation, since the free acid concentration

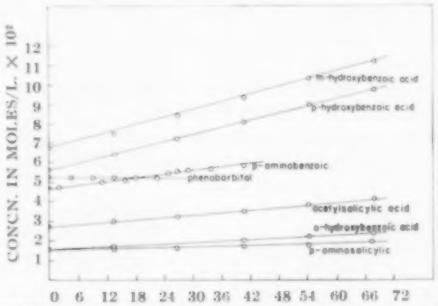

Fig. 1.—Phase diagram showing the effect of varying concentrations of dimethylurea on the apparent solubility of several organic acids in water at 30°.

in the system is an invariant. However, data presented here does offer a means of comparison of the extent of complex formation of the various pharmaceuticals under investigation.

Interactions with Polyethylene Glycol.—The effect of the polyethylene glycol on the apparent solubility in water at 30° of several compounds under study is represented in Figs. 2 and 3. It is seen that *p*-aminobenzoic acid and methyl and propyl *p*-hydroxy benzoate do exhibit a definite reaction as would be expected on the basis of a previously proposed theory (1). Sorbic acid exhibited little tendency to complex, whereas boric acid did not interact with the polyethylene glycol. This negative interaction may probably be attributed to

the high hydrophilic nature of boric acid. As with the dimethylurea interactions, no attempt was made to calculate stability constants for reasons already mentioned.

GENERAL DISCUSSION

Data have been presented indicating complex formation between sym-dimethylurea and polyethylene glycol with a number of pharmaceutical compounds. Although the information presented here does not permit calculation of exact stability constants for these reactions, it does provide a qualitative means of comparison of the degree of interaction of the various complexing agents under study. It also indicates that reactions of this type are far more widespread than commonly realized. Consideration of such interactions in formulation of drug preparations should be recognized. It was for this reason that a study of the parabens and polyethylene glycol was undertaken. Evidence has been presented, Fig. 2, indicating that methyl

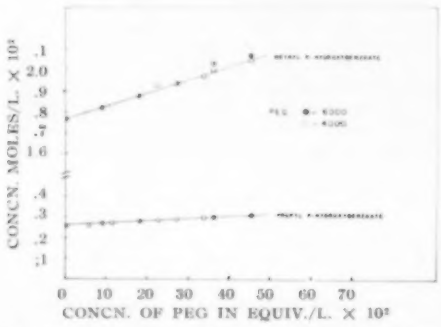

Fig. 2.—Influence of PEG 4000 and 6000 on the solubility of the parabens in water at 30°.

and propyl *p*-hydroxy benzoate do undergo molecular formation with PEG. The effect of this interaction on the antibacterial activity of the parabens is being studied in our laboratory.

PROCEDURE

Reagents.—Recrystallized *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-aminosalicylic acid, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, acetylsalicylic acid, boric acid, sorbic acid, and phenobarbital; 0.05 *N* barium hydroxide solution, 0.1 *N* alcoholic sodium hydroxide solution, bromothymol blue, and thymol blue as indicators; sym-dimethylurea and polyethylene glycol 4000 and 6000.

Method of Analysis.—Excess quantities of the compounds under investigation were placed in 125-ml. glass-stoppered bottles together with varying concentrations of dimethylurea or polyethylene glycol. Fifty milliliters of distilled water was added to each, except for the hydroxybenzoic acids, to which was added 50 ml. of 0.005 *N* sulfuric acid solution in order to suppress any ionization. The bottles were placed in a mechanical shaker in a constant-temperature bath and equilibrated for a period not less than ten hours at 30° after which

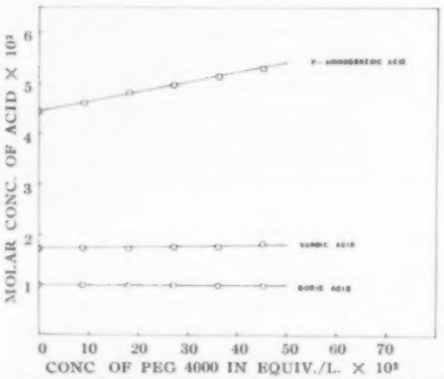

Fig. 3.—Phase diagram showing the effect of varying concentrations of PEG 4000 on the apparent solubility of certain acids in water at 30°.

time aliquot portions of the supernatant liquid were removed and analyzed for acid content by titration. *p*-Aminobenzoic acid was titrated with barium hydroxide solution, using bromothymol blue as the indicator. The remaining acids, except boric acid in which the U. S. P. XV method was used for analysis, were titrated with alcoholic sodium hydroxide solution, using thymol blue as the indicator. The quantity of the acids used were as follows: *p*-aminobenzoic acid 0.5 Gm., *p*-hydroxybenzoic acid 1.0 Gm., *o*-hydroxybenzoic acid 0.4 Gm., *m*-hydroxybenzoic acid 1.0 Gm., *p*-aminosalicylic acid 0.2 Gm., phenobarbital 0.3 Gm., boric acid 5.0 Gm., and sorbic acid 1.0 Gm. Both methyl and propyl *p*-hydroxybenzoate were determined spectrophotometrically at a wavelength of 256 mμ, correction being made for the very small absorption due to the polyethylene glycol. The quantity of methyl and propyl *p*-hydroxybenzoate used per 50 ml. distilled water was 0.4 and 0.2 Gm., respectively.

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Ultrasonic Depolymerization of Natural Polymers*

By W. F. HEAD, JR.,† and W. M. LAUTER‡

The action of 275 kc. ultrasonic waves on several naturally occurring gums and one natural material, partially modified by synthesis, has been studied. Methods were given for the interpretation of solution viscosities in terms of molecular weight decrease. Possible oxidative influences on the depolymerizations were also investigated and specific rate constants were calculated.

THE INFLUENCE of ultrasonic energy on emulsions, dispersions, and suspensions has been reported in the literature on several occasions (1–6). Beal and Skauen have called attention to the fact that proper selection of the emulsifying or suspending agent is important when these preparations are made in this manner (1).

Through true depolymerization, causing irreversible viscosity decrease, or by temporary destruction of structural viscosity, a much less efficient emulsifying or suspending aid might result. If however, the micellar structure of the gum or other agent in question is more highly hydrated by ultrasonic treatment, leading to an increase in viscosity, this material should then prove more effective in its use. It also seems reasonable to expect that combinations of these processes may occur simultaneously, although not necessarily to the same degree.

With sufficient data concerning the molecular weight or viscosity decrease of a gum as the time of insonation increases, a prediction of the best time of irradiation required to produce the most stable emulsion or suspension would be possible since these products are usually obtained quite rapidly by ultrasonic means. For example, tragacanth has been shown in this work to exhibit a maximum viscosity after approximately twenty minutes whereas at the end of two and one-half hours viscosity is very low. If a suspension of some material, using tragacanth as the suspending agent, is desired, it might be well to investigate a twenty-minute ultrasonic treatment.

If the depolymerization of an emulsifying agent is not too rapid, a stable emulsion could still be produced, provided the time of irradiation is not too long. It has been shown that continued exposure to ultrasonic waves actually increases the particle size in emulsions (3).

* Received May 15, 1957, from the College of Pharmacy, University of Florida, Gainesville.

† Fellow, American Foundation for Pharmaceutical Education.

Present address: Eli Lilly & Company, Indianapolis, Ind.

‡ Professor of Pharmaceutical Chemistry, University of Florida.

The authors are indebted to Dr. Harold M. Beal, College of Pharmacy, University of Connecticut, for the construction of the ultrasonic transducer used in this work and to Mr. Hugh Wise, Jr., Department of Chemistry, University of Florida, for the illustration of the apparatus.

The depolymerization of various natural and synthetic gums (7–10), proteins (11–14), and synthetic polymers (15–18) has been studied previously at different frequencies and under different conditions. In general, the viscosity or molecular weight of the substance decreases to a limiting value at which time no further change occurs.

Schmid (19) and Sobue and Ishikawa (20) have proposed equations for determining the rate of depolymerization or viscosity decrease; however, these expressions require the use of constants specific for each polymer. Chambers (13) has also reported insonation time *versus* activity curves for pepsin in the audible sound range and he represents the loss of peptic activity by a standard first-order kinetic equation.

The predominant cause of these phenomena is thought to be brought about by cavitation. The large resistance of a polymer molecule to movement, compared to a vibrating solvent molecule under the influence of ultrasonic action, introduces a frictional force which has been calculated (17) as being approximately five times the force necessary to break a carbon-to-carbon or carbon-to-oxygen bond. When the polymer molecule reaches some lower molecular weight it offers less resistance to movement, frictional forces decrease, and further depolymerization ceases. Weissler (21, 22) reports that the violence of cavity collapse may be involved. This would seem to increase the vibrational friction. Other cavitational effects, such as local heat up to several hundred degrees, electrical discharge, and oxidation may also contribute to depolymerization.

EXPERIMENTAL METHODS AND MATERIALS

Aqueous solutions of the gums used in this investigation were exposed to ultrasonic waves of 275 kc. frequency for varying periods of time, allowed to stand for twenty-four hours, and their viscosities taken at 37°. The twenty-four hour storage was considered necessary in order to allow the return of structural or anomalous viscosity to an equilibrium, thereby giving a more accurate representation of the true depolymerization.

These viscosities were then interpreted in terms of

the molecular weight of the substance according to the following equations (17): Relative viscosity − 1 = Specific viscosity; Specific viscosity × C = Molecular weight, where C is a constant characteristic for each gum. If the average molecular weight and the specific viscosity of the gum is known prior to ultrasonic treatment, C may be calculated. This value may then be used with viscosities obtained after irradiation for determination of molecular weight decrease.

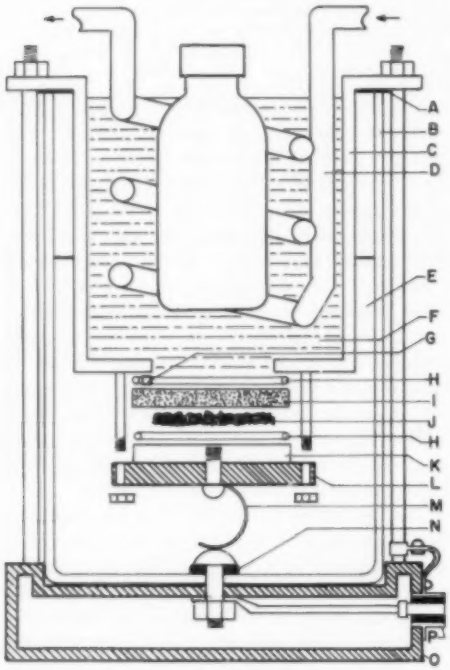

Fig. 1.—Ultrasonic transducer. A—rubber gasket; B—6 in. x 8½ in. Pyrex jar; C—metal housing; D—copper cooling coil; E—transformer oil; F—irradiation chamber; G—aluminum foil contact; H—rubber gasket; I—barium titanate crystal; J—copper wool contact; K—solid brass plate; L—composition material; M—metal contact; N—rubber gasket; O—composition base; P—amphenol plug.

With the molecular weight data thus obtained, specific rate constants were calculated using the following expression: log (M.W.^{0}/M.W.^{t}) = (Kt/2.3) where M.W.^{0} and M.W.^{t} are the molecular weights originally and after t minutes of insonation. Flory (23) has pointed out another method of treating these data, however the same constants are obtained by either method.

In some of the samples a peculiar, sharp odor, similar to ozone, was noticed after approximately thirty minutes' treatment. Therefore, all work was duplicated with samples which had been previously deaerated by passing nitrogen into them for twenty minutes. In this way, possible oxidative influences on the mechanism of the depolymerization were studied.

Irradiations were carried out in a one-pint, flexible polyethylene bottle and samples were withdrawn at various time intervals with a pipet. A copper cooling coil immersed in the irradiation chamber was used to prevent the temperature from rising above 40°. A Rich-Roth ultrasonic generator, model 400, and a specially constructed transducer, utilizing a flat disk barium titanate crystal, were used in this work. The details of the transducer are shown in Fig. 1. This unit, operating at 275 kc., delivered 124 watts of acoustical energy as determined calorimetrically.

EXPERIMENTAL RESULTS

Acacia.—One and two per cent solutions of acacia were treated ultrasonically for periods of time up to two and one-half hours, however, no significant viscosity change was observed. This confirms the results obtained by Beal and Skauen (1). It has been pointed out that with concentrated solutions the structural aggregates of a polymer are too strong to be affected by ultrasonic waves (24). The concentrations used here were thought advisable, however, in view of the low initial viscosity of acacia solutions. The results obtained with deaerated samples were also negative.

Viscarin®.[1]—This product, primarily a sodium carrageenate, has a molecular weight of approximately 200,000 (25). One-half per cent solutions were irradiated up to two and one-half hours and a logarithmic depolymerization was observed. These results are shown in Fig. 2. Samples containing only dissolved nitrogen depolymerized at an even faster rate as seen from the specific rate constants in Table I.

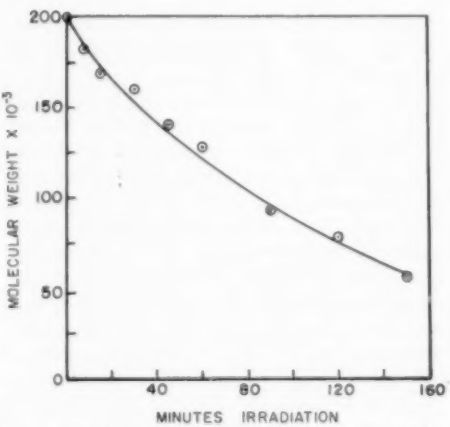

Fig. 2.—Ultrasonic depolymerization of Viscarin.

Agar.—One-quarter per cent solutions were treated as before and viscosity decrease was noted during the first forty minutes. Past this point, however, the viscosity of the samples increased.

[1] Algin Corporation of America, New York, N. Y.

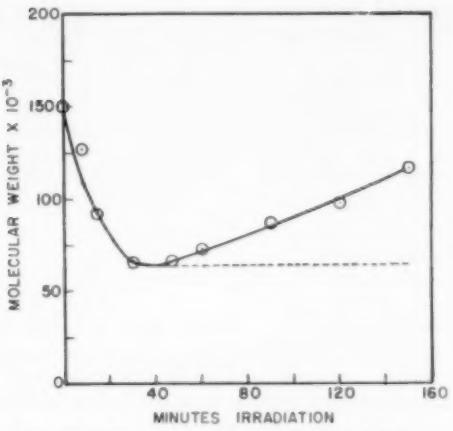

Fig. 3.—Ultrasonic depolymerization of Agar.

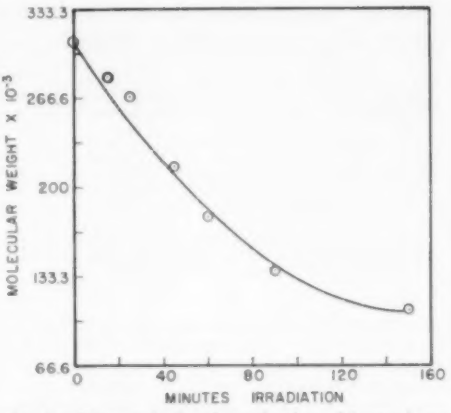

Fig. 4.—Ultrasonic depolymerization of locust bean gum.

This would appear to be an increase in molecular weight with increased time of irradiation, however, it is thought that this increase in viscosity is brought about by increased micelle-hydration or by dispersion of gum colloids allowing more efficient wetting and that the depolymerization process has reached a limiting value at forty minutes. These results are shown in Fig. 3, the extrapolation representing the depolymerization plateau. In order to test the validity of this plateau, molecular weight determinations by an osmotic pressure method were made on original agar samples, and samples which had been exposed to ultrasonic energy for forty minutes and two and one-half hours. As was expected, the samples that had been irradiated showed no significant deviation in molecular weight and the unexposed agar samples were considerably higher.

Samples which had been saturated with nitrogen showed a similar behavior, although the rate was slightly slower. For purposes of rate constant calculations, the data up to forty minutes, the point at which depolymerization ceased, were used. The molecular weight of agar is proposed to be in the range of 150,000 (26, 27).

Locust Bean Gum.—One-half per cent solutions of this gum, which is reported to have a molecular weight of 310,000 (28), were irradiated up to two and one-half hours. A depolymerization curve, as seen in Fig. 4, very similar to Viscarin was obtained. With samples treated with nitrogen the rate of depolymerization also increased. The specific rate constants are shown in Table I for both untreated and nitrogen treated samples.

Tragacanth.—The depolymerization curve of 0.5% tragacanth solutions, irradiated up to two and one-half hours, resembles that of agar in being atypical. However, in this case the anomaly is reversed in that a viscosity increase is observed initially, followed by a decrease. Since tragacanth is composed of two fractions, soluble tragacanthin and insoluble bassorin, it appeared possible that ultrasonic solubilization of bassorin could account for the viscosity increase.

With this in mind, a 1.25% solution of tragacanth, equivalent to 0.5% tragacanthin, was prepared,

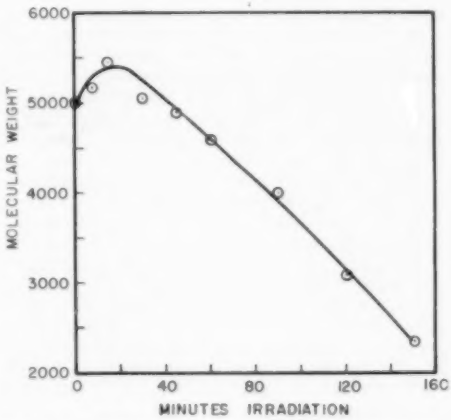

Fig. 5.—Ultrasonic depolymerization of tragacanth

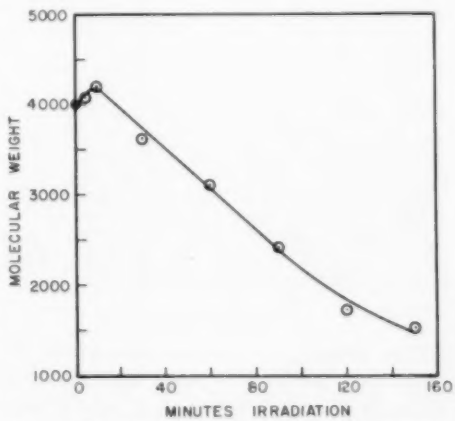

Fig. 6.—Ultrasonic depolymerization of tragacanthin.

TABLE I.—CHARACTERISTICS AND ULTRASONIC DEPOLYMERIZATION SPECIFIC RATE CONSTANTS OF VARIOUS GUMS

Gum	Mol. Wt.	Type Polymer	$K(O_2)^a$	$K(N_2)^b$	Reaction Order
Acacia	250,000	Complex	...	...	...
Viscarin	200,000	Linear	8.2×10^{-3}	1.7×10^{-2}	First
Agar	150,000	Linear	2.9×10^{-2}	1.3×10^{-2}	First
Locust Bean	310,000	Linear	8.1×10^{-3}	2.7×10^{-2}	First
Tragacanth	5,000	Complex	4.5×10^{-3}	1.7×10^{-2}	Zero
Karaya	9,500,000	Complex	...	...	...
4,000 c. p. s. Methocel[®][c]	140,600	Linear	3.7×10^{-2}	9.7×10^{-2}	First

[a] Specific rate constants of samples containing dissolved air, expressed in min.$^{-1}$.
[b] Specific rate constants of samples containing dissolved nitrogen, expressed in min.$^{-1}$.
[c] Dow Chemical Company, Midland, Mich.

centrifuged and the tragacanthin decanted from the bassorin portion. This solution was irradiated as before. It was observed that the initial viscosity increase was suppressed markedly; however, the remainder of the depolymerization curve remained approximately the same. These results are seen in Figs. 5 and 6.

Tragacanth also appears to be unusual in its order of reaction (depolymerization). While the other gums in this study follow first-order kinetics, tragacanth and tragacanthin fit a zero-order reaction. In calculating the specific rate constants for tragacanth, the data from twenty minutes to two and one-half hours were used. As before, samples treated with nitrogen were examined and results similar to those obtained without treatment were obtained. These specific rate constants appear in Table I.

Molecular weight data for tragacanth is varied and conflicting. In this investigation an average of 5,000 for tragacanth and 4,000 for tragacanthin were used (29, 30).

Karaya.—One per cent solutions of karaya gum, containing dissolved air and nitrogen were irradiated up to two and one-half hours. However, since these solutions, like acacia, are not very viscous, and because the molecular weight-viscosity relationship is not accurate for a molecular weight as high as 9,500,000 (28), no significant viscosity or molecular weight changes were observed.

Methocel®.—The method of determining the molecular weight change of Methocel was somewhat different from the other gums. Information was supplied which enabled us to obtain the molecular weight of the entire viscosity series of Methocels (31). One-half per cent solution viscosities of 15 c. p. s., 100 c. p. s., 400 c. p. s., 1,500 c. p. s., and 4,000 c. p. s. grades of Methocels were plotted *versus* their molecular weights. The 0.5% 4,000 c. p. s. Methocel was then irradiated up to two and one-half hours, allowed to stand twenty-four hours, and the viscosities taken. From the molecular weight-viscosity curve, changes in molecular weight could be obtained. A logarithmic depolymerization was observed which exhibited a slightly faster rate when the sample was deaerated with nitrogen. These results are shown in Fig. 7 and Table I.

SUMMARY

From Table I, it is interesting to note that Viscarin, agar, locust bean gum, and Methocel, which are all linear polymers in the same general range of molecular weights, have first-order specific rate constants which are comparable. Acacia, also in this molecular weight range, is, however, not linear but more compact and does not depolymerize. Tragacanth, a polymer of much lower molecular weight and complex structure, follows zero-order kinetics. Karaya, a much larger, more complex polymer, does not seem to depolymerize.

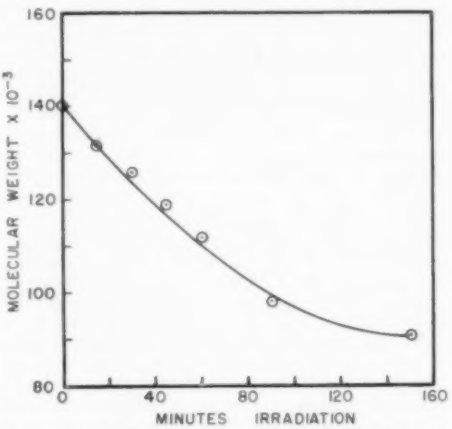

Fig. 7.—Ultrasonic depolymerization of Methocel.

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A Colorimetric Assay for Chlorobutanol*

By C. R. REHM and W. J. MADER

A colorimetric assay has been developed for the routine determination of chlorobutanol in parenteral drug preparations. The method is based on the formation of a colored complex between ferric ion and the hydroxamic acid derivative of chlorobutanol resulting from the reaction of chlorobutanol with hydroxylamine in alkaline solution. The color formation obeys Beer's law over the range of 0.1 to 0.8 mg. of chlorobutanol per ml. of sample solution. Optimum conditions for the color formation have been determined and interferences are discussed.

CHLOROBUTANOL (1,1,1-TRICHLORO-2-METHYL-2-PROPANOL) is a commonly used preservative in parenteral drug preparations. The concentration employed for the purpose of preservation against bacterial growth is of the order of 0.5 per cent. Although a number of methods exist for the determination of this compound, they are all based on alkaline hydrolysis followed by the titration of a hydrolysis product (1–3) or, by the titration of the excess alkali remaining after hydrolysis (4). These methods, while relatively accurate, are rather time consuming and inconvenient for routine determinations.

Apparently little or no work has been done on colorimetric methods of determining chlorobutanol. Passerini, *et al.* (5), reported the formation of a violet color from heating chlorobutanol (and other polyhalogenated compounds) in alkaline solution with sodium nitroprusside and adding a trace of ferric chloride. While the authors claimed the method to be very sensitive, no applications to quantitative measurements were made. As a result of the present study, a rapid and convenient colorimetric method has been developed for the routine determination of chlorobutanol. The method is particularly advantageous since only a few milligrams of chlorobutanol are required.

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EXPERIMENTAL

Reagents.—The following reagents were used:

Hydroxylamine HCl, 1 *M*—Dissolve 7.0 Gm. of hydroxylamine HCl in distilled water and dilute to 100 ml. This solution is stable for several weeks if kept refrigerated.

Sodium Hydroxide, 3.5 *M*—Dissolve 14 Gm. of reagent grade sodium hydroxide in distilled water and dilute to 100 ml.

Hydrochloric Acid, 3.5 *M*—Dilute 29 ml. of reagent grade hydrochloric acid to 100 ml. with distilled water.

Ferric Chloride, 0.3 *M* in 0.1 *M* HCl—Dissolve 8.0 Gm. of reagent grade ferric chloride hydrate in 0.1 *M* HCl and dilute to 100 ml. with same solvent.

Standard Chlorobutanol Solution, 0.5 mg. per ml.—Dissolve 100 mg. of anhydrous chlorobutanol (or 105 mg. of the hemihydrate) in distilled water and dilute to 200 ml. Prepare fresh daily.

Procedure.—In the absence of interfering substances (see discussion), dilute the sample with distilled water so that a concentration of 0.1 to 0.6 mg. of chlorobutanol per ml. is obtained. Into separate glass-stoppered test tubes pipet 4 ml. of the sample solution, 4 ml. of the standard chlorobutanol solution, and 4 ml. of distilled water. To each test tube add 1 ml. of hydroxylamine HCl solution and 1 ml. of sodium hydroxide solution, swirling the contents of the tubes after each addition. Allow the tubes to stand at room temperature (25° ± 2°) for 20 minutes. Then add to each tube 1 ml. of hydrochloric acid and 1 ml. of ferric chloride solution, again swirling the contents of the tubes after each addition. Allow the tubes to stand for 5 minutes, then shake vigorously and loosen stoppers to permit the

dissolved gas to escape or bubble a fine stream of air through the solutions for 30 to 60 seconds. Determine the absorbance of the sample and standard solutions against the blank solution at 500 mμ in 1-cm. cells in a suitable spectrophotometer.

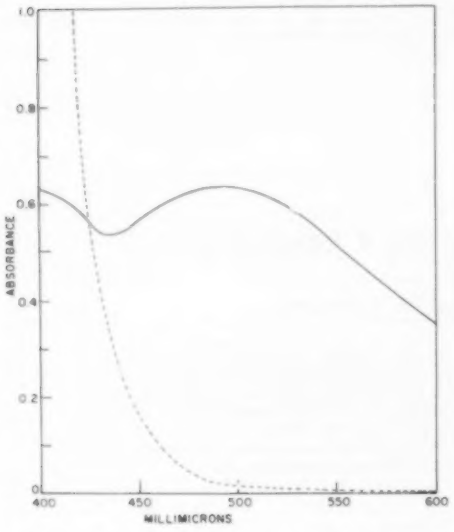


Fig. 1.—Absorption spectra of complex of ferric chloride-hydroxamic acid derivatives of chlorobutanol and of ferric chloride blank solution.
——Spectrum of colored complex against ferric chloride blank solution.
– – – Spectrum of ferric chloride blank solution against water.

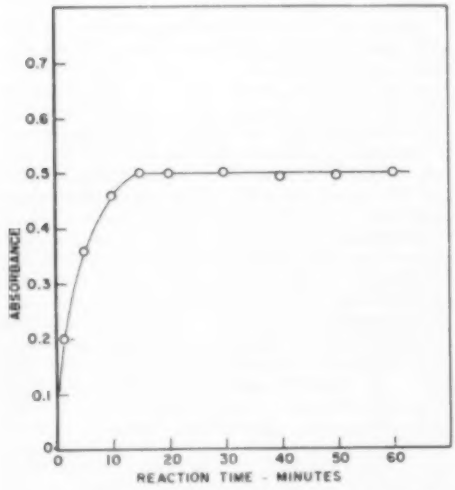


Fig. 2.—Plot of absorbance of final solution against time of reaction. Initial concentration of chlorobutanol was 0.5 mg./ml.

Where nonvolatile interfering substances are present in the sample, steam distil the chlorobutanol from a 3- or 4-ml. quantity of the sample solution in a suitable micro steam-distillation apparatus (see discussion). Add one drop of concentrated sulfuric acid to the sample, prior to distillation, and distil for 6 minutes. Rinse down the condenser with distilled water and add the washings to the distillate. Dilute the distillate with distilled water so that the concentration of chlorobutanol is in the range of 0.1 to 0.6 mg. per ml. and proceed as before, using a 4-ml. sample for assay.

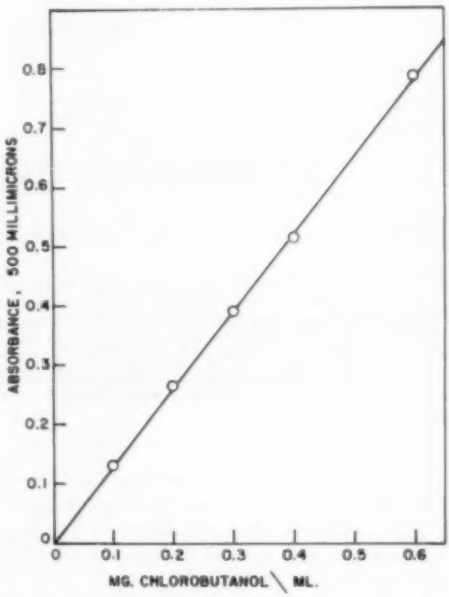
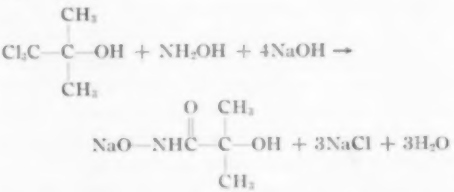


Fig. 3.—Plot of absorbance of final solution against initial concentration of chlorobutanol.

Calculation.—Mg. chlorobutanol/ml. = $A_{sample}/A_{standard} \times 0.5 \times$ dilution factor.

DISCUSSION

The reaction between chlorobutanol and hydroxylamine in alkaline solution can be expressed by the equation:



The absorption spectrum of the colored complex formed between ferric ion and the hydroxamic acid in acid solution is characterized by a broad maximum at 500 mμ as seen in Fig. 1. Although the

absorption of the blank solution at this wavelength is significant, it is small and reproducible. It was interesting to note that similar colored complexes were obtained with chloral and bromal. Although elevated temperatures were required to produce the hydroxamic acid derivative of these compounds, a similar colorimetric method for the determination of these compounds should be possible.

In order to determine the optimum reaction time for the formation of the hydroxamic acid, the absorbance of the final solution as a function of reaction time was investigated. From the curve shown in Fig. 2, it can be seen that at room temperature, a constant maximum absorbance is obtained after 15 minutes. On this basis, 20 minutes was selected for the assay procedure. Since the reaction time at room temperature is convenient, the use of elevated temperatures was not investigated.

A plot of color intensity *versus* concentration of chlorobutanol, as shown in Fig. 3, was found to be linear over a wide range of concentration. Although similar absorbance values were obtained for different runs using the same concentration of chlorobutanol, there were significant differences indicating that a standard must be run with each series. The colored complex is not completely stable and fades at the rate of 5% per hour. However, by reading the absorbance values at a timed interval after the addition of the ferric chloride solution, error due to this effect is obviated.

TABLE I.—COLORIMETRIC ASSAY OF CHLOROBUTANOL SOLUTIONS

Chlorobutanol, mg./ml. Solution	Found by Assay, mg./ml.[a]	Found by Assay After Steam Distillation, mg./ml.[a]
10.00	10.14	9.88
10.00	9.95	9.99
5.00	4.92	4.90
5.00	5.04	5.02
1.00	1.01	0.99
1.00	1.00	0.99

[a] Assay results based on standard chlorobutanol solution of 0.500 mg./ml. Samples were diluted approximately to this concentration prior to assay.

In Table I are shown typical results obtained with known aqueous solutions of different concentrations of chlorobutanol. Results obtained before and after distillation of the chlorobutanol from the sample solutions indicate that recovery by distillation is essentially 100% and that the precision of the method is ±2%.

Interferences.—The method is subject to a number of interferences. The presence of other compounds such as esters, imides, acid chlorides, and acid anhydrides which form hydroxamates would, of course, give high results (6). The presence of anions, such as tartarate or citrate which complex with ferric ion, or salts, such as phosphate and phthalate which form precipitates with ferric ion, also interfere in the method. Since the majority of interfering compounds which might be present in parenteral fluids are nonvolatile, quantitative separation of the chlorobutanol by steam distillation is easily carried out in most instances. An all glass micro steam-distillation apparatus consisting of a boiler flask, sample flask of 20-ml. capacity, and a 4-inch straight bore condenser, was found to be very satisfactory. A trap between the sample flask and condenser is necessary to prevent spray carryover. By limiting sample volumes to 3 or 4 ml., quantitative distillation of the chlorobutanol can be carried out in five to six minutes.

SUMMARY

1. A colorimetric method for the determination of chlorobutanol has been developed. The absorbance of the final solution at 500 mμ is linearly dependent upon the chlorobutanol concentration.

2. Optimum conditions for color development have been determined and interferences are discussed.

3. Separation of the chlorobutanol from nonvolatile interfering substances prior to assay is easily effected by steam distillation.

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Erroneous Values for Sodium and Potassium Content of Rat Tissues Dry-Ashed in Pyrex*

By M. KATHLEEN CARTER† and MARGARET E. GREIG

It has been found that dry ashing rat tissues in Pyrex glass containers at 550° is not a reliable procedure for the preparation of such tissues for sodium and potassium analyses.

THE OBJECT OF THIS PAPER is threefold: to compare the results of three methods commonly used in the preparation of tissues for sodium and potassium analyses, to point out the high degree of error involved in one of the methods, and to suggest a probable interpretation for this error.

METHODS

Homogenization with Trichloroacetic Acid (TCA). *A*, Dried tissue samples—About 200-mg. samples of several tissues from male Wistar or Sprague Dawley rats were removed immediately after the animals were decapitated and exsanguinated. A sample of each tissue was placed in a weighed beaker and dried at 110° for at least five hours. After obtaining a dry weight, a small amount of water was added to the beaker. After allowing the dried tissue to soften in the water, the tissue and water were transferred quantitatively to a glass homogenizer. The pestle used was made of Teflon. A ground glass pestle could not be used since the friction of two glass surfaces would grind sodium off into the solution and introduce an error in the sodium determinations. TCA (25% solution) was added directly to the homogenized tissue-water suspension and the mixture rehomogenized. The final homogenate, 10 ml., consisted of the tissue suspended in 5% TCA. After shaking several times during a thirty minute period, the suspension was centrifuged for fifteen minutes at 2,000 r. p. m. (International Centrifuge), and the supernatant fluid was then decanted.

B, Fresh tissue samples—The same procedure as given under *A* was followed, except that a fresh tissue sample was homogenized immediately after a wet weight was obtained. The final homogenate, 10 ml., was a 2% suspension of fresh tissue in 5% TCA.

Dry Ashing in Platinum.—Tissue samples (about 200 mg. each) were placed in platinum crucibles, dried at 110° for several hours, and the crucibles were then placed in an electric muffle furnace at 550° for two hours. The ash remaining from each of the tissue samples was dissolved in distilled water sufficient to make 10 ml. total solution.

Dry Ashing in Pyrex.—The same procedure was followed as given above except that wet and dry weights were obtained and the ashing was carried out for two and three hour periods in 15-ml. Pyrex beakers.

The supernatant fluids of all samples were analyzed for sodium and potassium concentration using the Beckman DU Spectrophotometer with flame attachment. Corrections were made for the interference of sodium on potassium readings and for the interference of potassium on sodium readings.

TABLE I.—SODIUM AND POTASSIUM CONTENT OF SEVERAL TISSUES OF THE WISTAR RAT PREPARED FOR ANALYSIS BY DIFFERENT METHODS

Tissue[a]	Homogenized with TCA—Wet Tissue (±S. D.)	Homogenized with TCA—Dried Tissue ±(S. D.)	Ashed Tissues (Pyrex Beakers) (3 Hours, 550°) (±S. D.)
Milliequivalents of Potassium/Kilogram Wet Tissue			
Kidney	70.5 (0.71)	72.2 (0.89)	35.1 (4.85)
Heart	76.8 (0.27)	77.0 (3.28)	37.8 (7.25)
Liver	86.7 (2.59)	89.2 (1.12)	48.0 (4.78)
Brain	92.8 (3.52)	96.3 (2.93)	48.1 (8.31)
Milliequivalents of Sodium/Kilogram Wet Tissue			
Kidney	50.2 (3.05)	49.6 (3.26)	72.1 (8.62)
Heart	34.5 (1.97)	34.7 (1.50)	61.9 (1.85)
Liver	21.6 (1.54)	21.2 (1.70)	27.8 (3.92)
Brain	38.3 (2.21)	39.0 (2.33)	74.1 (6.00)

[a] Tissues from six normal male Wistar rats weighing from 280–300 Gm. Each value represents the average of six samples.

TABLE II.—SODIUM AND POTASSIUM CONTENT OF SEVERAL TISSUES OF THE SPRAGUE DAWLEY RAT PREPARED FOR ANALYSIS BY DIFFERENT METHODS

Tissue[a]	Homogenized with TCA, Wet Tissue (Range)	Ashed Tissues (Pyrex Beakers) (2 hours, 550°) (Range)
Milliequivalents of Potassium/Kilogram Wet Tissue		
Kidney	73.2 (71.1–75.2)	38.5 (34.2–40.8)
Heart	76.7 (74.4–79.4)	43.8 (37.8–52.8)
Liver	88.6 (86.3–91.6)	61.2 (56.4–66.0)
Brain	94.3 (91.4–96.0)	51.3 (44.0–54.7)
Milliequivalents of Sodium/Kilogram Wet Tissue		
Kidney	55.0 (52.8–57.0)	73.1 (67.8–78.2)
Heart	38.6 (34.9–42.3)	59.7 (55.9–63.8)
Liver	23.0 (22.2–23.7)	27.6 (21.8–35.0)
Brain	45.0 (43.8–46.8)	72.2 (69.6–74.4)

[a] Tissues from two normal male Sprague Dawley rats weighing 280 Gm. Each value represents the average of four samples, two from each rat tissue.

Correction was also made for the effect of 5% TCA on potassium readings.

RESULTS AND DISCUSSION

The results for the sodium and potassium content of the rat tissues prepared by the above three procedures are presented in Tables I, II, and III.

* Received April 27, 1957, from the Research Division, The Upjohn Company, Kalamazoo, Mich.

† Present address: Department of Pharmacology, Tulane University School of Medicine, New Orleans 12, La.

TABLE III.—SODIUM AND POTASSIUM CONTENT OF SEVERAL TISSUES OF THE WISTAR RAT PREPARED FOR ANALYSIS BY DIFFERENT METHODS

Tissue[a]	Homogenized with TCA, Wet Tissue	Ashed Tissues (Platinum Crucibles) (2 hours, 550°)
Millequivalents of Potassium/Kilogram Wet Tissue		
Kidney	81.0	82.3
	82.3	80.2
		79.1[b]
		80.6[b]
	81.6 (Av.)	80.5 (Av.)
Heart	79.6	82.0
	84.8	86.1
	82.2 (Av.)	84.1 (Av.)
Milliequivalents of Sodium/Kilogram Wet Tissue		
Kidney	58.4	57.6
	59.8	62.2
		58.4[b]
		58.4[b]
	59.1 (Av.)	59.2 (Av.)
Heart	38.7	37.3
	39.3	38.2
	39.0 (Av.)	37.8 (Av.)

[a] Tissues from two normal male Wistar rats weighing 380 Gm. Values are for individual samples.
[b] Platinum crucibles with covers.

The sodium and potassium content of tissues (fresh or dried) homogenized with 5% TCA agreed with the values obtained from the tissues ashed in platinum crucibles. These values also check with the majority of values reported in the literature (1). However, dry ashing of tissues in Pyrex beakers at 550°, gave low values for the potassium content and high for the sodium content.

The interpretation offered to explain the discrepancy in this last method is that, at the high temperature used, some of the potassium in the tissue was taken up by the glass and some of the sodium in the glass came out into the tissue sample. Potassium was not lost through volatilization since the values for potassium content in those tissue samples ashed in platinum crucibles, with or without covers, were essentially the same as those values when the tissue samples were homogenized with TCA.

Apparently, the decrease in potassium content is not limited to ashing in Pyrex glass only, as Wilde (2) noted that tissues ashed in Vycor (Corning Glassware) were lower in potassium content than tissues ashed in platinum crucibles.

These data demonstrate that dry ashing tissues in Pyrex glass containers for two or three hours at a temperature of 550° is not a reliable procedure for the preparation of tissues for the analysis of sodium and potassium.

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Notes

A Note on Paper Chromatography of Some Quaternary Ammonium Salts*

By WILLIAM E. ROSEN, VIRGINIA P. TOOHEY, and ANTHONY C. SHABICA

Quaternary salts of isoindolines, tetrachloroisoindolines, and related systems have been examined by paper chromatography, and some generalizations concerning their behavior are noted.

FOLLOWING the discovery of the ganglionic blocking activity of 4,5,6,7-tetrachloro-2-methyl-2-(2-trimethylammonioethyl)-isoindolinium dichloride (1) (Ecolid®), a considerable amount of chemical work was carried out (2) on tetrachloroisoindolines and related systems. The need for a rapid analytical tool for the examination of mixtures led to the use of paper chromatography. A study of the R_f values subsequently accumulated suggested several generalizations concerning the chromatographic behavior of quaternary ammonium salts and of certain related tertiary amines.

All chromatograms were carried out on Whatman No. 1 filter paper using the ascending method. The developing solvent was *n*-butanol saturated with 0.1 *N* ammonia, and the spray reagent was a modified Dragendorff solution (3).

RESULTS

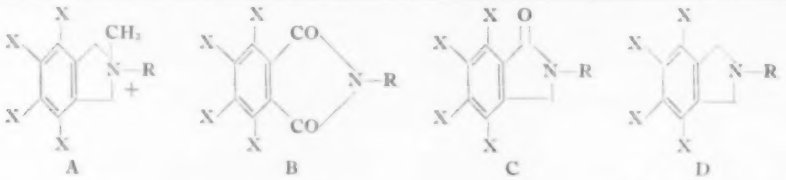
Table I lists R_f values in increasing numerical order (i. e., in decreasing order of relative solubility in the stationary water phase) according to type compound.

DISCUSSION

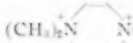
The monomethohalide salts gave spots more sharply defined and more intense than the tertiary

* Received March 22, 1957, from the Research Department, CIBA Pharmaceutical Products Inc., Summit, N. J.

TABLE I.—R_f VALUES OF AMMONIUM SALTS AND AMINES



A B C D

Compound	X	R	Anion(s)	R_f Values
A	H	$CH_2CH_2N^+(CH_3)_3$	$2\,Cl^-$	0.05
			$2\,I^-$	0.13
A	H	CH_2CH_2Cl	Cl^-	0.34
A	H	CH_2CH_2Br	Cl^-	0.38
			Br^-	0.43
A	Cl	$CH_2CH_2N^+(CH_3)_3$	$2\,Cl^-$	0.16
			$2\,Br^-$	0.23
			$2\,I^-$	0.25
A	Cl	CH_3	Cl^-	0.34
			Br^-	0.55
			I^-	0.61
A	Cl	CH_2CH_2Br	Cl^-	0.53
			Br^-	0.64
A	Cl	$CH_2CH_2N(CH_3)_2$	Br^-	0.73
A	Cl	$CH_2CH_2N^+H(CH_3)_2$	$2\,Br^-$	0.73
A	Cl	H	Cl^-	0.92
			Br^-	0.93
[A]$_2$	Cl	CH_2	$2\,Br^-$	0.53
A	3 Cl, 1H	$CH_2CH_2N^+(CH_3)_3$	$2\,I^-$	0.20
B	H	$CH_2CH_2N^+(CH_3)_3$	I^-	0.14
B	H	$CH_2CH_2N(CH_3)_2$	...	0.52
B	Cl	$CH_2CH_2N^+(CH_3)_3$	I^-	0.42
B	Cl	$CH_2CH_2N(CH_3)_2$	...	0.70
C	Cl	$CH_2CH_2N(CH_3)_2$	...	0.96
D	H	$CH_2CH_2N(CH_3)_2$	...	0.89
D	Cl	$CH_2CH_2N^+(CH_3)_3$	Cl^-	0.55
			Br^-	0.55
			I^-	0.62
D	Cl	CH_3	...	0.96
D	Cl	$CH_2CH_2N(CH_3)_2$	...	0.98
	...	...	$2\,Cl^-$	0.00
			$2\,TsO^-$	0.00
$(CH_3)_3N^+—(CH_2)_6—N^+(CH_3)_3$	...	...	$2\,Cl^-$	0.02
$(CH_3)_4N^+$	...	...	Cl^-	0.08
			Br^-	0.11
$BrCH_2CH_2N^+(CH_3)_3$	...	...	Br^-	0.22
$CH_3NHCH_2CH_2N^+(CH_3)_3$	...	...	I^-	0.24
$C_6H_5CH_2N^+(CH_3)_3$	...	...	Cl^-	0.33

amines, but less so than the dimethohalide salts. Hydrohalide salts, which were usually indistinguishable by melting point from the methohalide salts, chromatographed like their corresponding amines. The quantity of material chromatographed affected the R_f value. A large excess of dimethohalide overran the normal spot and gave high observed values (doubling the amount increased the R_f value 5–10%). In contrast, excess monomethohalide trailed the normal spot and gave low observed values.

Any factor which increased the solubility of the compound in water lowered the R_f value. An oxygen-containing polar function such as the imide group lowered the R_f value relative to the corresponding isoindoline. Introduction of chlorine on the benzene ring increased the R_f value. With a series of salts, the R_f values were $Cl^- < Br^- < I^-$; the effect of anions on R_f values of quaternary ammonium salts of this type has already been reported (4). The R_f value of a monoquaternized diamine was higher when the isoindoline, rather than the terminal nitrogen was quaternized; this is undoubtedly related to the hydration behavior of these salts (2). Small changes in room temperature or the presence of impurities had little, if any, effect on R_f values.

Another bismuth spray reagent (5) was tried and found to be less satisfactory for the monohalide salts.

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A Note on the Use of Sorbitol Solution in Pharmaceutical Formulations*

By A. J. BARTILUCCI, A. DiGIROLAMO, and H. EISEN

Evidence is presented to indicate the significance of the shipping containers for commercial sorbitol solutions used in the formulation of liquid dosage forms. Some inconsistency in stability data of ascorbic acid formulations is attributed to the relatively high trace metal content of the sorbitol solution used. This solution was supplied in a poorly protected metal container.

THE STABILITY of ascorbic acid in vehicles containing a commercial sorbitol solution has been reported by several investigators (1, 2).

An inconsistency in the stability data of ascorbic acid in this vehicle was observed during a formulation study of certain vitamin combinations. This was made apparent by the inability to reproduce results of work previously done in this and other laboratories. As a result of a critical investigation, certain observations were made. These are offered as a probable explanation for the discrepancies found in our laboratory during the current formulation study.

Visual examination was made of a sorbitol solution that had been standing at room temperature for a prolonged period in a metal container having a pour spout and snap-on closure with resilient liner and suitable facing. The stored solution had acquired a pale amber color. In addition, the inner surface of the spout exhibited evidence of attack by the solution.

As a result of the observed undesirable change in the solution, an additional quantity of sorbitol solution was purchased from a wholesale distributor. This was received in a metallic container of the type previously described. The statement, "Distributed by ————," appeared on the label, thus inferring possible subdivision by the company named from a larger bulk quantity of material.

An additional quantity of sorbitol solution was received in a glass container having a plastic screw-cap closure with a plastic resilient liner.

Separate dilutions of varying concentration were prepared from each of the sample solutions of sorbitol, using glass-distilled water as the diluent. About 50 ± 3 mg. of ascorbic acid U. S. P. was dissolved in the original solution and in each dilution.

Typical storage results after six months at 40° included the following: a 70% w/w sorbitol solution of ascorbic acid exhibited a loss of 18.3% in ascorbic acid content when prepared from sorbitol solution received in a metal container, as compared to a 5.4% loss when prepared with the sorbitol solution received in a glass container.

A solution prepared by dissolving ascorbic acid in a solvent consisting of equal parts of sorbitol solution and glass-distilled water was found to have lost 51.8% of its ascorbic acid content when prepared with the sorbitol solution received in a metal container, as compared to a 26.6% loss when prepared with the sorbitol solution received in a glass container.

The rapid destruction of ascorbic acid in the sorbitol solution stored in the metal container was thought to be due to the presence of metal contaminants resulting from the solvent effect of this vehicle on the container itself. The presence of the metal contaminants was confirmed by spectrographic analysis[1] of the two samples of sorbitol solution. This analysis showed that the sorbitol solution stored in the glass container had 0.25 mcg. of nickel, 1 mcg. of iron, and 1.3 mcg. of unidentified heavy metals per gram of sample, or 2.55 p. p. m. total trace metals; while the sorbitol solution stored in the metal container had 3.3 mcg. of nickel, 11 mcg. of iron, and 2.4 mcg. of unidentified heavy metals per gram of sample, or 16.7 p. p. m. total trace metals.

It is interesting to note the relationship between these findings and the maximum trace metal limit of 10 p. p. m. permitted by the N. F. X and the specifications of one supplier with maximums of 5 p. p. m. of nickel, 3 p. p. m. of iron, 1 p. p. m. of lead, and 1 p. p. m. of arsenic.

Consideration, therefore, should be given to the possibility of unwittingly employing contaminated solutions in the development of liquid formulations containing materials which are affected by trace quantities of such heavy metals. This contamination may arise from improper plant storage and handling, as well as during shipment.

It is not the intent of this note to discredit the usefulness of sorbitol solution in pharmaceutical formulations, particularly those containing ascorbic acid. The use of sorbitol solution as a suitable vehicle for ascorbic acid already has been reported by many investigators, including those previously cited. This information is similar to the now common knowledge of the possibility of introducing metal contaminates by glycerin which is stored in metal containers (3).

[1] The assistance of Dr. C. I. Jarowski and Mr. D. Manley of Chas. Pfizer & Co. in supplying the spectrographic data is gratefully acknowledged.

REFERENCES

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(3) Husa, W. J., "Pharmaceutical Dispensing," Husa Brothers, Iowa City, Iowa, 1951, p. 466.

* Received May 3, 1957, from the Research Laboratory, St. John's University College of Pharmacy, New York, N. Y.

Presented to the Scientific Section, A. PH. A., New York meeting, April–May, 1957.

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The United States Pharmacopœial Convention, Inc.

SEVENTH ANNUAL FINANCIAL STATEMENT

Based upon the Report of the Auditor for the year ended December 31, 1956

STATEMENT OF INCOME AND EXPENSE

Income:		
Sales of pharmacopeias:		
Collections	$134,072.60	
Less—Decrease in accounts receivable	27,424.85	
		$106,647.75
Sales of reference standards and reprints:		
Collections	$ 25,743.73	
Less—Decrease in accounts receivable and adjustment of prior years' collections	973.49	
		24,770.24
Interest on investments, less amortization of bond premiums of $22.65		6,839.85
Interest on savings deposits		5,141.21
Use of text by others		2,640.00
Miscellaneous		985.72
Total Income		$147,024.77
Less—Expense:		
Revision	$106,967.24	
Less—Increase in inventory	1,705.31	
		$105,261.93
Administration		6,631.47
Publications	$ 44,714.02	
Less—Increase in inventory	11,481.25	
		33,232.77
Headquarters	4,838.68	
Add—Depreciation expense		
Building	$2,430.77	
Furnishings and equipment	3,227.07	5,657.84
		10,496.52
Total Expense		$155,622.69
Excess of Expense over Income		$ 8,597.92

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS

Receipts:	
Sales of pharmacopeias	$134,072.60
Sales of reference standards and reprints	25,743.73
Interest on investments	6,862.50
Interest on savings deposits	3,326.61
Use of text by others	2,640.00
Miscellaneous	985.72
Total Receipts	$173,631.16

	Revision	Administration	Publications	Headquarters	Furnishings and Equipment	Total
Expense:						
Printing & binding			$44,399.62			$ 44,399.62
Salaries	$ 49,729.88	$5,313.99		$1,218.60		56,262.47
Honoraria	29,450.00*					29,450.00
Meetings	2,923.93	541.36				3,465.29
Supplies	1,673.31	70.62				1,743.93
Postage & telegrams	1,632.54	50.00				1,682.54
Utilities				2,174.50		2,174.50
Repairs & maintenance				1,445.58		1,445.58
Furnishings & equipment					$2,963.33	2,963.33
General	21,557.58†	655.50	314.40			22,527.48
Total Expense	$106,967.24	$6,631.47	$44,714.02	$4,838.68	$2,963.33	$166,114.74

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Book Notices

Microchemical Journal. Vol. I, Issue 1. Issued semiannually by the Metropolitan Microchemical Society. Interscience Publishers, Inc., New York. Price $9.60 per year.

This journal, a new publication under the auspices of The Metropolitan Microchemical Society, is designed to obtain a wider distribution of the philosophy, ideas, and techniques of microchemical procedures as applied to all areas of the physical and biological sciences. The editorial board includes: N. D. Cheronis, J. A. Means, A. G. Mistretta, T. S. Ma, A. A. Benedetti-Pichler, and Bella Rosenfeld.

Technique of Organic Chemistry. Vol. X. *Fundamentals of Chromatography.* By HAROLD G. CASSIDY. Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., New York, 1957. xvii + 447 pp. 15 x 23 cm. Price $9.75.

The earlier discussions on chromatography by the author of this volume dealt with Definitions, Molecular aspects of adsorption, Measurement of adsorption and treatment of data, Relations between relative adsorbability and properties of phases, Relations between relative extent of adsorption and properties of adsorptive, and Graded eluents and adsorbents, and were included in Vol. V of this series [see THIS JOURNAL, **40,** 303(1951)]. The present volume does not repeat the earlier material but deals with principles of chromatography abundantly illustrated with detailed examples of applications.

Starting with a discussion on the Nature of chromatography, Vol. X takes up: Molecular interactions on which chromatographic separations rest, General theory, Gas-liquid partition chromatography, Column partition chromatography, Paper partition chromatography and some closely related methods, Adsorption chromatography, Ion exchange, Electron exchange polymers, Foam and emulsion fractionation, On recognizing and evaluating zones, On the relation of R or R_f to molecular structure, On choosing mobile and stationary phases, and On using chromatography. An appendix gives the symbols used and the sources of equipment. The 1042 references in the book are given at the end of the text, as is the general subject index. The style, format, and type of this volume follows the others in this excellent series. The book is a valuable addition to this rapidly growing field.

Methods of Biochemical Analysis. Vol. IV. Edited by DAVID GLICK. Interscience Publishers, Inc., New York, 1957. ix + 362 pp. 15 × 23 cm. Price $9.50.

This is the fourth annual review of methods and techniques of biochemical analysis. The earlier

three volumes were reviewed in THIS JOURNAL, **43,** 445(1954); **44,** 385(1955); and **45,** 510(1956).

Volume IV contains discussions by selected contributors on: Determinaton of carotene, Determination of vitamin A, Measurement of polyunsaturated fatty acids, Determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, The pH-stat and its use in biochemistry, Assay of sulfatases, Determination of serum acid phosphatases, Determination of amino acids by use of bacterial amino acid decarboxylases, and Determination of succinic dehydrogenase activity. In addition to references for each chapter, author and subject indexes for volume IV and cumulative author and subject (titles of reports) for volumes I–IV are appended. This volume in a very useful series continues the excellent style, format, and binding of the earlier volumes.

Dextran and Its Use in Colloidal Infusion Solutions. By ANDERS GRONWALL. Academic Press Inc., New York, 1957. 156 pp. 14 × 21.5 cm. Price $4.

This monograph, by one of the two Swedish men who introduced the colloidal infusion solution of dextran, is divided into the following chapters: Production and chemistry of undegraded dextrans, Partial depolymerization of dextrans and properties of depolymerization products, Adaptation of molecular dimensions of dextrans to the desired therapeutic effect, On the fate of intravenously administered dextran, Serology of dextrans, Hemodynamic and shock therapeutic effect of dextran solutions in animal experiments, and Clinical investigations with dextran solutions.

References are given after each chapter but no general index is included. The brief text includes much interesting information; although one might have difficulty in finding it without an index.

Symposium on Arteriosclerosis. Bull. schweiz. Akad. med. Wiss. Vol. XIII, Special Number (April, 1957). Benno Schwabe and Company, Basel, Switzerland, 1957. vii + 488 pp. 16 x 24 cm.

After the opening lecture on ways of managing arteriosclerosis, the symposium was divided into three sections: Blood-vessel factors and arteriosclerosis (13 papers), Metabolism and arteriosclerosis (24 papers), Clinic and therapy (7 papers). Each paper is published in the language in which it was presented, but each has a summary in English, French, and German. Notes from the discussions are appended. An author index to these papers is included.

Light, Vegetation and Chlorophyll. By J. TERRIEN, G. TRUFFAUT, and J. CARLES. Translated by MADGE E. THOMPSON. Philosophical Library, Inc., New York, 1957. 288 pp. 12 x 18.5 cm. Price $6.

This book is a translation of two French publications: *Lumière et végétation* (Light and vegetation), the first, is concerned with light as a form of energy and its utilization by plants; and *L'Energie Chlorophyllienne* (Chlorophyll and Energy), the second, deals with the chemistry of chlorophyll and photosynthesis. The text is interesting and well written, and is a good compilation of diversified yet related information in this field. A more complete index would be helpful for reference to specific items.

The Chemistry of Organometallic Compounds. By EUGENE C. ROCHOW, DALLAS T. HURD, and RICHARD N. LEWIS. John Wiley & Sons, Inc., New York, 1957. vi + 344 pp. 15 x 23 cm. Price $8.50.

This book includes recent developments in its discussions on structure and physical properties, preparation, reactions, and applications of organometallic compounds. The size of the book has been restricted by the authors' critical selectivity of topics and examples, and their realistic limitation of the discussions on Grignard reagents. The text material is organized on the basis of the general properties of the carbon-metal bond; electronegativity and bond polarity have been used as indicative of the type of organometallic compound to be expected in a given instance. After discussing general factors related to organometallic compounds and presenting the theory of the carbon-metal bond and the methods of preparation, the compounds are taken up in the order of the elemental groups I through VII. Concluding chapters take up the transition metals, organometallic compounds in organic synthesis, and special types of compounds. References are given throughout the text and author and subject indexes are appended. The book should be useful to graduate students and research workers in this field.

British Pharmaceutical Codex 1954 (1957 Supplement). Edited by the CODEX REVISION COMMITTEE. The Pharmaceutical Press, London, 1957. xii + 124 pp. 14 x 22.5 cm. Price £1.7*s.*, 6*d.*

This Supplement includes the new monographs added to the British Pharmacopoeia by the Addendum 1955; it lists the monographs that were transferred from the Codex 1954 [see review in THIS JOURNAL, **44,** 190(1955)] to the B. P., gives modifications of B. P. monographs with changes in formulas, and indicates deletions from the Codex 1954. A monograph on reserpine includes as the assay a direct nonaqueous titration in glacial acetic acid with 0.05 *N* perchloric-acetic acid using solution of quinaldine red as indicator. Reserpine tablets are assayed by centrifuge extraction of the powdered sample with chloroform and measurement of the extinction of a 1-cm. layer of the solution at 268 mμ.

An interesting development of nomenclature is indicated in the monograph title "Sodium Calciumedetate" for the calcium chelate of the disodium salt of ethylene diamine NNN'N'-tetraacetic acid, which is referred to in the literature as disodium calcium ethylene diaminetetraacetic acid or disodium calcium EDTA, or as the ethylene diaminetetraacetate which is now indicated officially as the ED(e)TA(te). The usual excellent work of the Codex Revision Committee is evident in this Supplement

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